The Importance of Norovirus and Cadmium in Shellfish and Implications to Human Health

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Abstract

Shellfish are an important food source however they are known to harbour bacteria, viruses and toxic chemicals that can be detrimental to their human consumers. Oysters have been associated with the gastroenteritis virus Norovirus. New Zealand has some of the highest cases of foodborne illness in the western world.

This study investigated a possible link between periods of high rainfall and reported Norovirus outbreaks in four major cities in New Zealand (Auckland, Wellington, Christchurch and Dunedin) as well as national data. Norovirus is a highly infectious foodborne illness. Outbreaks of norovirus have been linked to the consumption of shellfish, and in particular oysters. Norovirus virions can enter the aquatic environment via sewage as a result of human shedding of the virus. This investigation into rainfall and Norovirus outbreaks found no statistically significant relationship, in a monthly or season setting.

In addition the relationship between environmental cadmium levels and exposure levels in New Zealand was investigated through meta-analysis. Cadmium is a heavy metal commonly associated with the mining of copper and zinc ores. It is found naturally in the environment, in air land and oceans Increased exposure to cadmium is known to have a number of serious detrimental health effects, in particular this study investigates cadmiums immunosuppressive properties.. Concentrations in New Zealand were compared with Canada, Italy and the UK to determine if New Zealand has a relatively high cadmium intake. Interestingly environmental levels (soil and oceanic) in New Zealand were low. Cadmium levels were higher in oysters than in mussels, with New Zealand oysters containing the highest concentration of cadmium presented. New Zealanders also had the highest cadmium burdens in the kidneys and the highest daily intakes. A No Observable Effect Level (NOEL) was calculated from mice data and compared to the daily intakes of the four countries. Both Canada and New Zealand were above this level.

Shellfish are a common mechanism for exposure to both Norovirus and cadmium. The levels of cadmium present in the diet of New Zealanders may be sufficiently high to suppress the immune system, making it more vulnerable to infections of enteric diseases such as Norovirus.

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Chapter 1: Norovirus Infection in New Zealand

1.1 Introduction to Norovirus

Norovirus is a leading cause of acute non-bacterial gastroenteritis worldwide (Donaldson *et al*., 2008; Siebenga *et al*., 2009; Patel *et al*., 2009). Belonging to the *Calciviridae* family of viruses, Norovirus is a small round non-enveloped single stranded, positive-sense RNA virus between 26 and 35nm (Buckow *et al*., 2008; Greening *et al*., 2009). Norovirus can be divided into five genogroups, GI-GV, with three genogroups primarily infecting humans (GI, GII and GIV). The other two genogroups primarily infect cows (GIII) and mice (GV). These genogroups can then be further divided into genoclusters (strains); Norovirus has over forty different strains (Donaldson *et al*., 2008).

Genogroups I and II, and the more than 25 different strains that comprise them, account for the majority of human Norovirus cases. Worldwide the most prominent strain in recent years has been GII.4 (Donaldson *et al*., 2008; Siebenga *et al*., 2009). With outbreaks of this cluster occurring much more frequently than any other strain within the GII genogroup and outbreaks of GI occurring even less frequently. In 1995-1996, US95/96, a subcluster of GII.4, accounted for approximately 55% of Norovirus outbreaks in the United States, and 85% in the Netherlands. Outbreaks of the US95/96 strain also occurred in Brazil, Australia, Canada, the United Kingdom, China and Germany (Noel *et al*., 1999; Donaldson *et al*., 2008). The US95/96 strain was subsequently replaced by two new variants between 2000 and 2004. In the United States, Farmington Hills became the dominant new strain, ultimately associated with approximately 80% of Norovirus outbreaks (Donaldson *et al*., 2008). In Europe, a new variant of GII.4, GII.4b proved to be the dominant strain. From 2004 the Hunter GII.4 variant (detected in Australia, Europe and Asia) became the dominant strain and in 2006 Hunter GII.4 was replaced by two new variants Sakai and Minerva. These two strains have played dominant roles in Norovirus outbreaks across the United States, Europe, and Asia since then (Donaldson *et al*., 2008).

Norovirus is highly infectious and prominent worldwide, and therefore it is perhaps surprising that our knowledge of human Norovirus biology is quite limited. This lack of knowledge is primarily due to a difficulty in culturing Norovirus using conventional virological techniques and a lack of small animal infection models in which to study Norovirus. In order to investigate and learn more about the biology of human Norovirus, Norovirus surrogates have been

employed. Common surrogates include poliovirus, feline calicivirus and more recently murine Norovirus (Cannon *et al*., 2006; Bae and Schwab, 2008). Much of the early work on Norovirus was conducted using sapoviruses as surrogates. However unlike Norovirus, feline calicivirus is transmitted by the respiratory route and not by food contamination (Buckow *et al*., 2008; Cannon *et al*., 2006). More recent studies have demonstrated that murine Norovirus (MNV-1) and human Norovirus share many biochemical and genetic characteristics (Bae and Schwab, 2008). They also share clinical symptoms and transmission routes in nature, primarily the faecal-oral route (Cox *et al*., 2009). More importantly, MNV-1 is the only known isolate among the five Norovirus genogroups to replicate in cell culture and in small animals e.g. mice (Bae and Schwab, 2008; Cannon *et al*., 2006). The use of MNV-1 has already provided many insights into the Norovirus biology. In recent studies murine Norovirus has been shown to replicate in dendritic cells *in vitro* and in macrophages *in vivo* and *in vitro* (Wobus *et al*., 2006). In a study by Wobus *et al* (2006) they successfully demonstrated MNV-1 ability to replicate readily in cell lines with a hematopoietic lineage, as well as in primary bone-marrow derived macrophages and dendritic cells. This will allow for more investigation of the biology of Norovirus in the future.

1.1.1 Norovirus and Humans

Norovirus is highly infectious, with only a few virions (virus particles) required for pathogenicity. It has an incubation period of between 24 and 48 hours, with clinical symptoms generally lasting 12-72 hours. Symptoms can include nausea, vomiting, abdominal cramps, myalgias and non-bloody diarrhea. While death is rare it can occur in individuals who are immune compromised or have other underlying health concerns (Donaldson *et al*., 2008; Greening *et al*., 2009; Patel *et al*., 2009).

Norovirus' ability to cause large outbreaks and secondary infection can be put down to two major features. Firstly, a relatively low infectious dose is required to cause infection generally as little as 10 virion particles are required for infection (Donaldson *et al*., 2008; Patel *et al*., 2009). The second reason is due to Noroviruses resilience to inactivation. While the majority of viruses are inactivated by drying, freezing, cooking and the use of disinfectants, Norovirus can survive such treatments (Patel *et al*., 2009).

The only known reservoir for human Norovirus is human faeces (Lees, 2000; Atmar *et al*., 2008; Patel *et al*., 2009). Transmission can occur via several routes, but faecal-oral is

considered the most important (Cox *et al*., 2009). Faecal pollution from sewage discharge, septic tank leachates and boat discharge can all cause contamination of shellfish beds, recreational waters and drinking water. Indeed faecal pollution is thought to be the key route for environmental contamination which can lead to human infection. Person to person transmission is one of the most common methods of dispersal for this virus in the community. Norovirus virions can become airborne and can stay active for long periods on hard surfaces that are not disinfected correctly. Infected humans also present an important reservoir for human infection, as viral shedding has been detected in samples from asymptomatic patients for up to three weeks post symptoms (Rockx *et al*., 2002). Norovirus is also thought to persist in the environment for around 2 months (Greening *et al*., 2003)

1.1.2 Norovirus, Sewage and Shellfish

Shellfish are an important food source for humans worldwide, and in New Zealand shellfish represent an important traditional food for Māori. Shellfish, particularly bivalves are also a well known potential carriers for a number of foodborne illnesses (Lees, 2000), including Norovirus. In part this is due to the method in which they feed and respire; by allowing water to flow over their gills they capture suspended particulate matter from the water (Figure 1.1). This particulate matter then passes into the mouth where it is either ingested or rejected as pseudo-faeces. Bivalves have the ability to filter considerable amounts of water, with oysters filtering between 10-20 litres of water per hour (Jorgensen, 1952). The large volumes of water they filter enable them to concentrate viral particles from their environment that far exceeds the levels found in the surrounding waters.

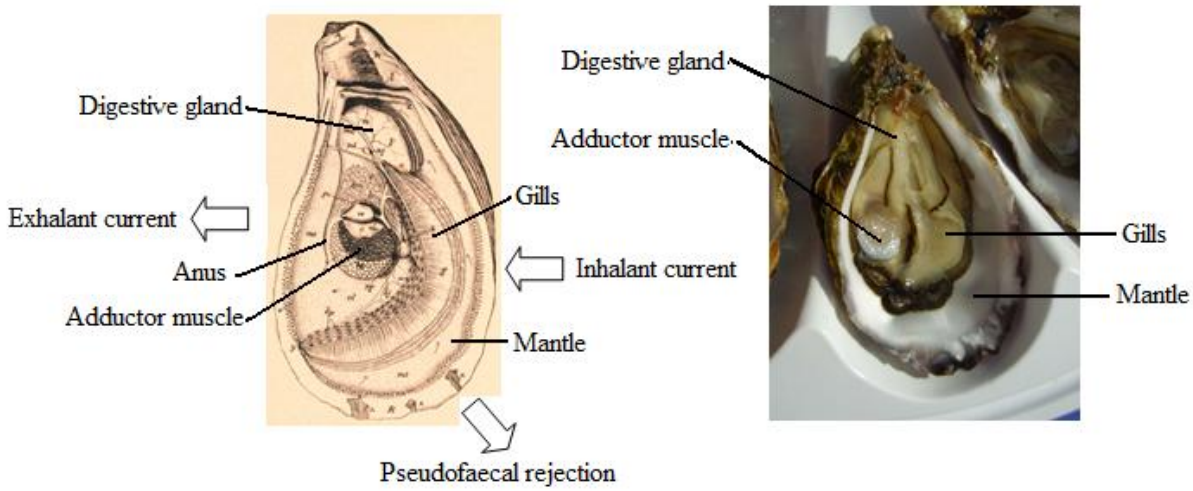

Figure 1.1 Anatomical diagram (left) and a photograph (right) of a oysters showing the organs involved in feed and respiration, and the areas of inhalation, exhalant and pseudo-faeces expulsion images sourced from
Left:http://commons.wikimedia.org/wiki/File:Oyster_anatomy.jpg
Right: http://en.wikipedia.org/wiki/File:Oyster.jpg

Viruses transmitted by the faecal-oral route like Norovirus, can be widely prevalent in the community and infected individuals shed millions of virions via faeces and vomit. Consequently these virions make their way into the sewage and areas where sewage is disposed such as waters surrounding outfall pipes and estuaries. It is not uncommon to find Norovirus present in aquatic environments that sewage is discharged into, even when sewage has been treated (see below) (van den Berg *et al*., 2005; Ueki *et al*., 2005; da Silva *et al*., 2007). Insufficient treatment of sewage can lead to an increase in the numbers of viruses being discharged. Many places worldwide have very limited or no treatment facilities for sewage and consequently semi-treated or raw sewage are pumped straight into aquatic environment. Sewage treatments can include waste stabilization ponds, bioreactors, clarifiers, oxidation and UV treatment (North Shore City Council, 2010; Wellington City Council, 2010b). A study in Japan investigated Norovirus which was detected in patients of gastroenteritis, domestic sewage, treated wastewater, river water and cultivated oysters. Treatment of sewage involved oxidation ponds and chlorination for disinfection before discharge in the Takagi River. Norovirus was present in 9/9 domestic raw sewage samples and 8/9 treated sewage samples between November 2003 and February 2004 (Ueki *et al*., 2005). From these results they concluded that Norovirus from human faeces arriving at the wastewater treatment plant was

insufficiently removed during the wastewater treatment process, and was discharged into the Takagi River.

There have been several incidences where Norovirus outbreaks have been linked to oysters, sewage and heavy rainfall. In Australia in 1990, 57 separate outbreaks over 18 days of viral gastroenteritis (believed to be Norovirus) were recorded (Bird and Kraa, 1992). Bird and Kraa (1992) traced the oysters back to the Sydney Rock oyster (*Saccostrea commercialis*) harvested from estuaries that were feed by the Georges River, which had been polluted as a result of heavy rainfall. After heavy rainfall a period of no harvesting was enforced and when water quality returned to normal, a further three days of no harvesting was allowed in order for the oysters to self cleanse, however this proved not to be long enough and resulted in infected oysters being sold to consumers who then became infected (Bird and Kraa, 1992).

1.1.3 Virus Inactivation in Shellfish

1.1.3.1 Depuration

Depuration (where shellfish are place in clean water to purge/cleanse themselves of sediments and toxins) has found to be an ineffective means of cleansing viruses from shellfish (Ueki *et al*., 2007; Nappier *et al*., 2008). Indeed, several Norovirus outbreaks have been associated with depurated shellfish. In the United Kingdom in 1985 after a lunch time reception where seafood was served, 13/51 people became ill with diarrhea, abdominal pain and vomiting (Heller *et al*., 1986). The most likely cause of the illness was thought to be Norovirus from contaminated oysters. The oysters were harvested from "grossly polluted waters" (Heller *et al*., 1986), and after an initial 72 hour depuration the oysters were placed in depuration tanks for a further 15 days. In addition a laboratory study conducted by Nappier *et al* (2008) they found that depuration was ineffective at removing several enteric viruses, including Norovirus, in the oysters *Crassostrea virginica* and *C. ariakensis*. These results clearly show that while depuration may be an effective method to allow shellfish to purge themselves of sediment and bacteria, it is inefficient at the removal of Norovirus.

1.1.3.2 Hydrostatic Pressure

Several studies have shown that high hydrostatic pressure may be the answer to inactivation of Norovirus particles in a commercial setting. High-hydrostatic pressure is known to reduce enzymatic activity (thus preserving the shellfish) and reducing the number of microorganism

present (He *et al*., 2002). High hydrostatic pressure generally involves placing the shellfish in bags and submitting them to high pressure. Pressures can vary, but good inactivation results have been reported by Kingsley *et al* (2007) at pressures from 300 to 450 MPa. Both Feline Calicivirus and Murine Norovirus have been inactive by this method, which is enhanced at temperatures below 20°C (Kingsley *et al*., 2007; Buckow *et al*., 2008). High hydrostatic pressure has the advantage that it can inactivate viruses while causing minimal changes in visual quality and taste (Kingsley *et al*., 2007; Buckow *et al*., 2008; Grove *et al*., 2008; Kingsley *et al*., 2007).

1.1.3.3 Cooking

The most common way of consuming shellfish is either raw or lightly steamed. However it has been shown that many conventional cooking techniques are unlikely to inactivate Norovirus present in shellfish (Hewitt and Greening, 2006). In this study Hewitt and Greening (2006) looked at Greenshell mussels (*Perna canaliculus*); they concluded that Norovirus was unlikely to be inactivated by steaming or boiling conditions within a reasonable domestic cooking time. Indeed, they found that after 300 seconds of steaming the internal temperature of the mussels had not reached the temperature necessary to inactivate Norovirus i.e. 90°C. While increasing the cooking time would inactivate Norovirus it did not reflect the conventional cooking practices. In mussels that were boiled until an internal temperature of 90°C for 90 seconds was reached and resulted in viral inactivation. The total time this took was 260 seconds (170 seconds was needed to reach an internal temperature of 90°C). However Hewitt and Greening (2006) found that after only 210 seconds all of the mussel shells had opened. On the basis that mussels are generally consumed when the majority of the shells have opened (indeed shell opening is used as an indicator of suitability to eat), it is unlikely that they have been cooking for a sufficient length of time to have maintained at a sufficiently high internal temperature to inactivate the virus.

1.1.4 New Zealands' Shellfish Industry

The New Zealand shellfish industry is an important source of food, both domestically and as an export product. Bivalves such as oysters (*Crassostrea gigas, Saccostrea glamerata,* and *Ostera Chilensis*) and mussels (*Perna canaliculus*) occur naturally in the majority of waters around New Zealand. Large quantities of Pacific oysters (*C. gigas),* and Greenshell mussels (*P. canaliculus),* are commercially farmed. They also represent the majority of our shellfish

exports. In 2009 mussels were the top export species for fish earning NZ$202 million (Seafood Industry Council, 2010). In 2008 mussels exported fresh or frozen weighed 32,038.6 tonne and oysters 122.0 tonne (Seafood Industry Council, 2009). It is therefore important to the New Zealand economy that New Zealand shellfish are free of viruses, including Norovirus, for fear of consumers being put off New Zealand shellfish if they suffer post consumption gastroenteritis.

1.1.4.1 Imported Shellfish and Norovirus Outbreaks

Imported shellfish represent a minor part of the New Zealand food supply; despite this imported oysters have been linked to a number of outbreaks in New Zealand. A 'famous' example is the outbreak of Norovirus at an international rugby game at Eden Park (Auckland, New Zealand), on the 17 June 2006 (One News, 2006; Simmons *et al*., 2007). Thirty percent of patrons in four hospitality areas reported illness, in one hospitality area alone 65% of patrons reported illness. The outbreak was caused by Korean Pacific oysters (*C. gigas*), instructions on the packet said they were to be cooked before consumption, however they were served raw. This was not the first outbreak of Norovirus linked to Korean oysters, early that year another three outbreaks occurred, one in Otago, one in the Hawkes Bay and one in Tauranga (Simmons *et al*., 2007). In 2004 a review of importation requirements for bivalve molluscan shellfish commenced (New Zealand Food Safety Authority, 2006; Greening *et al*., 2009), it resulted in 2006 in a change in conditions for importation. Exporting countries now have to demonstrated that bivalve molluscan shellfish have been derived from a regulated environment that manages hazards (e.g. Norovirus) and meets the New Zealand regulations (Animal Products (Regulated Control Scheme—Bivalve Molluscan Shellfish) Regulations 2006) (Greening *et al*., 2009). In 2008 limited trade in Korean oysters continued with a case by case basis.

1.1.4.2 New Zealand Shellfish and Norovirus Outbreaks

New Zealand oysters have also been linked to Norovirus outbreaks, in particular oysters from growing regions in and around Northland. In March 2001, Hong Kong refused entry of New Zealand Pacific oysters due to the high number of Norovirus outbreaks associated with oysters harvested from the Northland region (Greening *et al*., 2009). After several months, and Norovirus surveillance showing negative results from several exported batches of oysters Hong Kong re-allowed import of New Zealand oysters, with the exception of oysters grown in the Waikare Inlet. Waikare Inlet is has frequent contamination issues with sewage, however with improvements in septic tanks, sewage pipes, the sewage treatment plant and boating controls

has allowed the area to be reclassified as a "conditionally approved" site, from a restricted (Greening *et al*., 2009).

Many New Zealanders consume shellfish, and to many Māori shellfish are an important food sources. While shellfish are available in supermarkets many people, including Māori prefer to source their shellfish directly from the wild. It is these 'wild' shellfish that are at greater risk of containing bacteria and viruses, such as Norovirus, that may be detrimental to human health. These wild shellfish have a higher risk factor associated with them as they are unlikely to be in areas with strict water quality monitoring programmes, which are in place in areas of commercial farming.

1.2 Research Objectives

To study:

1) Norovirus outbreaks in the major cities in New Zealand.

2) The relationships between rainfall and human Norovirus gastroenteritis outbreaks in New Zealand

3) The occurrence of population outbreaks and environmental recording of Norovirus presence in shellfish in New Zealand

1.3 Methodology

This work comprises a meta-analysis of unpublished Norovirus outbreaks data from the Institute of Environmental Science Research (ESR) and published national rainfall data from the National Institute of Water and Atmospheric Research (NIWA). Environmental contamination in tuatua (*Paphies subtriangulata)* and cockles (*Austrovenus stutchburyi*) in the Christchurch region were supplied by the Christchurch City Council. The relationship between Norovirus outbreaks and rainfall was investigated. In addition the relationship between environmental contamination of shellfish and reported outbreaks in Christchurch was investigated.

1.3.1 Norovirus Data

Norovirus data were supplied by ESR from their National Surveillance Programme. Data on all reported outbreaks that occurred in the year 2000 and from 2005 until the end of 2009 were

included in this study. Data from 890 outbreaks were included. Data collected for Norovirus outbreaks included in the present study:

The date the outbreak was reported
The public health office the outbreaks was reported to
The number of cases involved in each outbreak
The geographic locations that the outbreak occurred in
The mode of transmission of Norovirus, modes included:
- Foodborne
- Waterborne
- Person to person
- Sexual contact
- Parental
- Environment
- Zoonotic
- Vectorborne
- Other mode
- Other mode specified
- Mode unknown

Setting that the outbreak occurred in
- Abattoir
- Café
- Camp
- Caterers
- Childcare
- Community
- Farm
- House
- Hospital acute care
- Hospital continued care
- Hostel
- Hotel/motel
- Other food setting
- Prison
- Rest home
- School
- Supermarket
- Swimming pool/spa
- Takeaway
- Tangi (Māori funeral service)
- Work place
- Other setting
- Other setting specified

Setting unknown

The investigation centered on the number of reported outbreaks rather than the number of individual cases in an outbreak. This was because a large number of outbreaks were reported in rest homes and hospitals where the numbers of individual people infected can reach large numbers and could potential skew the results – it is very likely that such outbreaks are due to a single source of infection.

The investigations looked initially at the four main centres, Auckland, Wellington, Christchurch and Dunedin (Table 1.1). Total New Zealand outbreak data were also included.

Table 1.1 Populations statistics of all ethnicities and Māori for Auckland, Wellington, Christchurch and Dunedin from the 2006 New Zealand Census.

	Total population	**Māori population (% of total population)**
Auckland	1,303,068	137,133 (11)
Wellington	448,956	55,437 (12)
Christchurch	521,832	36,669 (7)
Dunedin	193,803	12,270 (6)

1.3.2 Rainfall Data

Rainfall data were collected from NIWA's National Climate Database using the following below. Average rainfalls for Auckland, Wellington, Christchurch and Dunedin were calculated from selected rain stations in each region. Rainfall stations used were chosen based on relevance to the Health Districts used.

Information collected are as follows:

Data type used included:

Combined statistics calculated from observations and,

Monthly/annual statistic,

Rain type investigated was:

Total Rainfall

Weather stations used and locations:

See Table 1.2

Dates investigated were the year 2000 and from 2005-2009

Table 1.2 Number, name and location of weather stations used in the collections of rainfall data.

Region	Station Number	Station Name	Latitude	Longitude
Auckland	1412	Albany	-36.7496	174.7556
	2006	Pukekohe Ews	-37.2064	174.8638
Wellington	17029	Wallaceville Ews	-41.1354	175.0528
	25354	Wellington, Kelburn Aws	-41.285	174.768
	3394	Maungaraki No 2	-41.2121	174.8764
	3445	Wellington Aero	-41.322	174.804
	8567	Paraparaumu Aero Aws	-40.907	174.984
Christchurch	17244	Rangiora Ews	-43.3286	172.6111
	17603	Lincoln, Broadfield Ews	-43.6262	172.4704
	24120	Christchurch, Kyle St Ews	-43.5307	172.6077
	4843	Christchurch Aero	-43.0493	172.537
	4858	Christchurch Gardens	-43.531	172.619
Dunedin	15752	Dunedin, Musselburgh Ews	-45.90129	170.5147
	18437	Middlemarch Ews	-45.51814	170.1356
	18593	Ranfurly Ews	-45.1254	170.1003
	18594	Windsor Ews	-45.00829	170.8228
	5142	Oamaru Airport Aws	-44.973	171.081
	5323	Palmerston	-45.47554	170.7144
	5535	Lauder Ews	-45.0401	169.6842
	5893	Nugget Point Aws	-46.449	169.81
	7339	Dunedin Aero Aws	-45.929	170.197

Stations were chosen because of their location. If they contained complete data sets of all months of the years investigated (e.g.. 2000, and 2005-2009). The same stations were used each year, with the exception of;

Station 24120 in Christchurch was not used in 2000 but was included in the 2005-2009.

Station 25354 in Wellington was not used in 2000 but was included in the 2005-2009.

Stations 15752, 18437, 18593 and 18594 in Dunedin were not included in the data set for 2000, because of incomplete data sets.

After a preliminary screen of the data it was decided to exclude data from 2000 because the levels of reported Norovirus outbreaks were too few (e.g. Wellington had a total of 6 outbreaks,

with Christchurch and Dunedin having 4 and 1 respectively) for this year which would make outbreak/rainfall correlation statistically meaningless (figure 1.2).

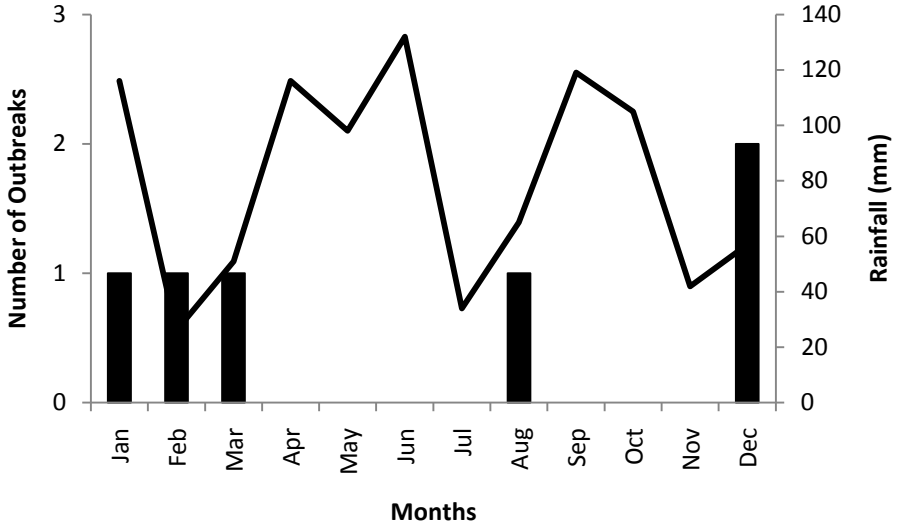

Figure 1.2 Norovirus reported outbreaks and average Rainfall for Wellington for 2000 showing that there were too few Norovirus outbreaks to conduct rainfall correlation statistics

1.3.3 Environmental Contamination

Norovirus contamination data for tuatua (*Paphies subtriangulata)* and cockles (*Austrovenus stutchburyi)* was kindly supplied by the Christchurch City Council. The data were collected as part of resource consent processes for a new sewage outfall pipe in Christchurch. These data were plotted against total monthly Norovirus outbreaks reported in the Christchurch region (data from ESR).

Tuatua for analysis were collected from:

Rockinghorse Road: Easting - 2490084; Northing – 5739348
Sumner Surf Club: Easting - 2490487; Northing – 5738089
Jellicoe Street: Easting - 2489232; Northing – 5741862

Cockles for analysis were collected from: shown in figure 1.3

Figure 1.3 Locations of cockle sampling points in the Avon-Heathcote estuary in Christchurch. Map kindly supplied by the Christchurch City Council

1.3.4 Analysis

Monthly and seasonally reported Norovirus outbreaks for Auckland, Wellington, Christchurch and Dunedin were plotted against average rainfall for their respective areas. In Christchurch reported Norovirus outbreaks were also plotted against

Correlation analysis was run using the statistical programme Minitab® version 16.1.0, (Minitab Inc, US) for monthly and seasonal data, to determine the relationship between rainfall and Norovirus outbreaks.

Cross-correlation analysis was also conducted with Minitab® for monthly data, with a lag of 1 month. A lag of 1 was chosen as rainfall may affect the following month, but was unlikely to affect any more than one month. While all lags are shown in the results tables, only -1 lags were considered possible, i.e. that rainfall in one month can affect reported Norovirus outbreaks in the next month. Lags that suggested that reported Norovirus outbreaks in one month could affect rain in the following month (1 lag) were not considered relevant to this study.

1.4 Result of Rainfall/Norovirus Correlation Studies

Norovirus from sewage enters the marine environment and is subsequently taken up by shellfish. Heavy rainfall can overflow the sewage treatment system which often cannot accommodate the huge increase in the volume of throughput. Which means, that untreated (albeit diluted) sewage enters the marine environment directly.

1.4.1 Monthly Outbreaks and Rainfall Data

Appendix 1 includes all of the raw data from the individual cities; total monthly outbreaks, supplied by ESR, and average rainfall calculated from the NIWA climate database.

1.4.1.1 Results for Auckland

Plots of rainfall and Norovirus outbreaks versus months clearly show the expected annual cycle of rainfall, but show that there is no relationship between Norovirus outbreaks and periods of high rainfall (Figure 1.4).

The total number of Norovirus outbreaks in a year increases from 2005 (33 outbreaks) to 2009 (80 outbreaks). In 2008 however there was a decrease in the number of reported Norovirus outbreaks; in 2007, 79 outbreaks were reported and in 2008 this dropped to 59 outbreaks. The total number of outbreaks recorded in 2009 (80) increase to be above the 2008 (59) levels and is similar to that of 2007 (79). There is a noticeable decrease in the number of outbreaks reported from August to December in 2008 (Figure 1.4).

There appears to be a correlation between rainfall and Norovirus outbreaks at specific times; in 2007 from May through till December, in 2008 from March through till October (Figure 1.4). However, only 2008 had a statistically significant correlation between average rainfall and Norovirus outbreaks (Table 1.3).

Cross correlation analysis shows a slightly significant lag effect in 2006 (Correlation coefficient = 0.450) (Table 1.4). In 2007 and 2008 correlations was stronger when there was no lag between outbreaks and rainfall (Correlation coefficients = 0.561 and 0.718 respectively).

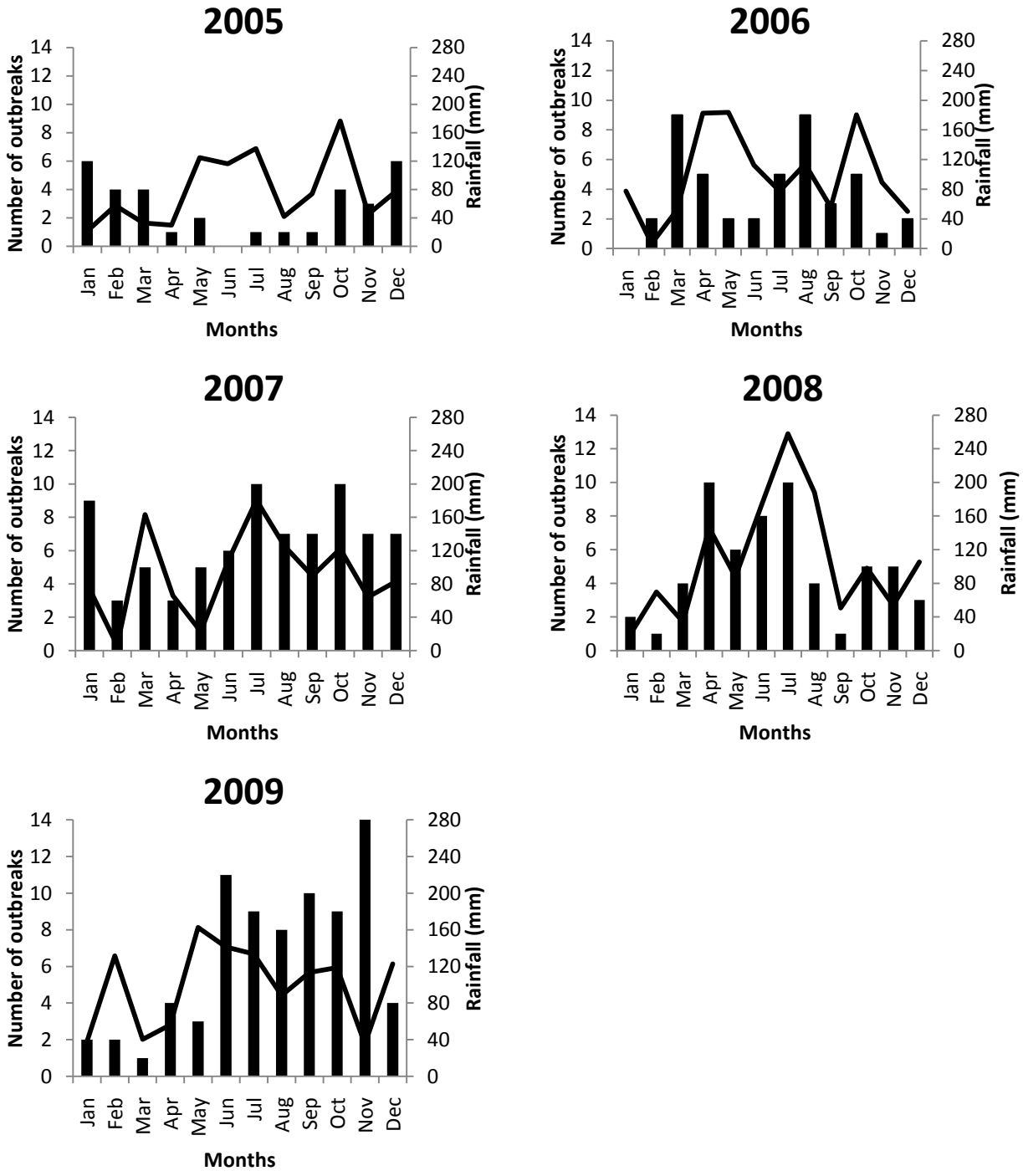

Figure 1.4 Relationship between total monthly recorded Norovirus outbreaks (bars) and average rainfall (line) for Auckland from 2005 -2009

Table 1.3 Correlation analyses between total monthly recorded Norovirus outbreaks and average rainfall in Auckland from 2005-2009

	Year				
	2005	2006	2007	2008	2009
Pearson Correlation	-0.214	0.123	0.561	0.718	-0.013
P-value	0.503	0.704	0.058	0.009	0.968

Table 1.4 Cross correlation analyses between total monthly recorded Norovirus outbreaks and rainfall in Auckland from 2005-2009. Lags: 1 outbreaks lag rainfall, lags, 0 no lag, -1 rainfall lags outbreaks.

Lag	Year				
	2005	2006	2007	2008	2009
1	-0.272	-0.623	0.05	-0.014	-0.507
0	-0.214	0.122	0.561	0.718	-0.013
-1	-0.536	0.450	-0.169	0.520	0.148

1.4.1.2 Results for Wellington

Plots of rainfall and Norovirus outbreaks vs. months show a variable annual cycle of rainfall, and no relationship between Norovirus outbreaks and periods of high rainfall (Figure1.5).

Wellington has the same increasing trend in the total number of reported outbreaks from year to year as Auckland with a decrease in 2008 (Figure 1.5). Interestingly, the increase in total number of outbreaks from 2008 to 2009 almost doubled, from 17 reported Norovirus outbreaks to 32. No statistically significant correlations between average rainfall and reported Norovirus outbreaks were found (Table 1.5).

The number of reported Norovirus outbreak per month is generally quite low, i.e. 3 outbreaks or less per month, from 2005-2008. However in 2009 May, June, July and August four or more Norovirus outbreaks are reported (Figure 1.5).

In 2009 from March until May there is an increase in rainfall. Also in 2009 from April to June there was an increase in Norovirus outbreaks possibly suggesting a possible lag effect. However there was no statistically significant lag for the year (Table 1.6).

The effect seen in 2008 (correlation coefficient = 0.400), is for a lag in rainfall, i.e. that the outbreaks in one month is correlated to the rainfall of the following month. The number of reported Norovirus outbreaks in one month cannot influence the amount of rainfall.

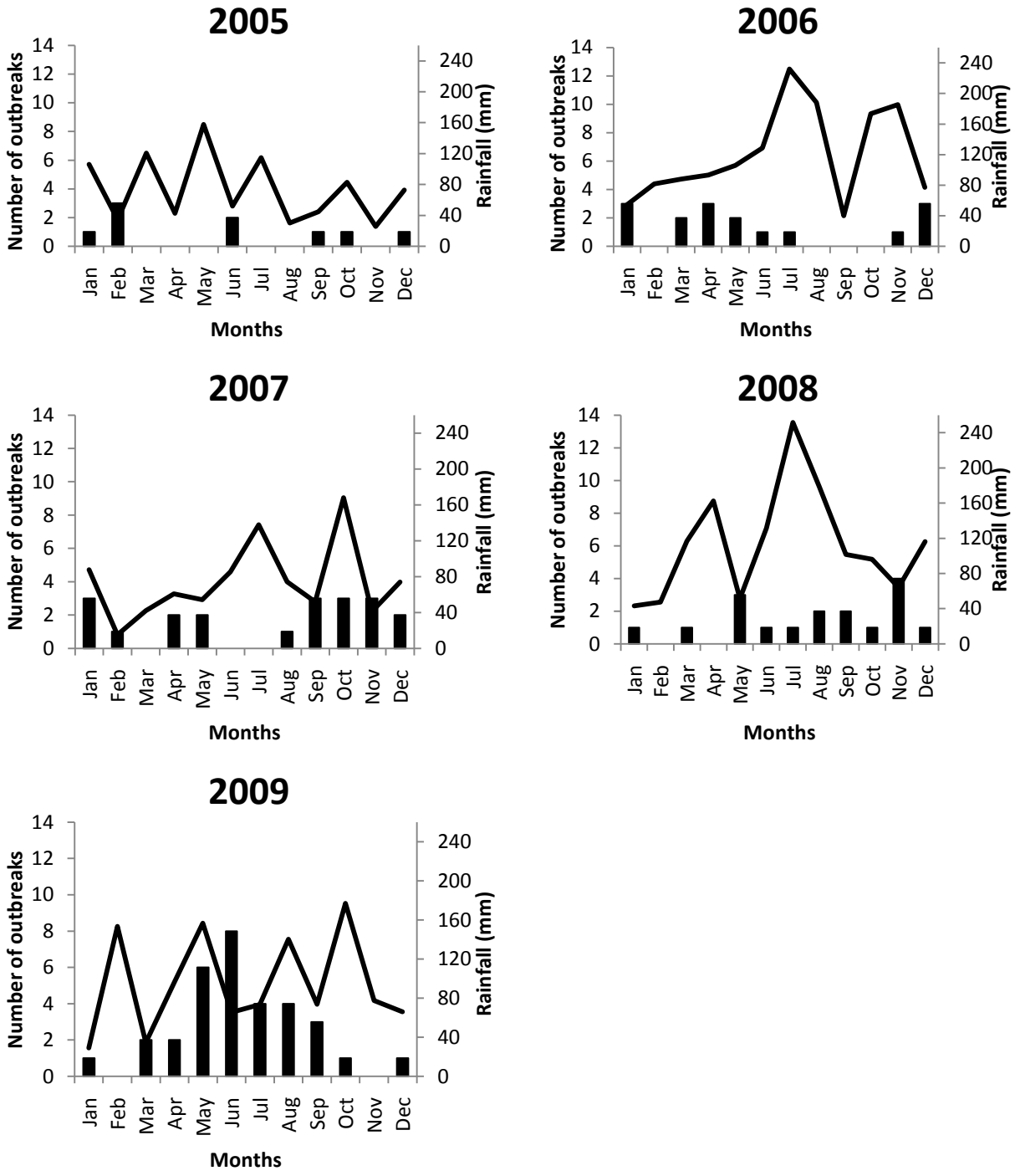

Figure 1.5 Relationship between total monthly recorded Norovirus outbreaks (bars) and average rainfall (line) for Wellington from 2005 -2009

Table 1.5 Correlation analyses between Norovirus outbreaks and rainfall in Wellington from 2005-2009

	Year				
	2005	2006	2007	2008	2009
Pearson Correlation	-0.331	-0392	0.047	-0.255	0.018
P-value	0.294	-.207	0.885	0.424	0.955

Table 1.6 Cross correlation analyses between total monthly recorded Norovirus outbreaks and rainfall in Wellington from 2005-2009. Lags: 1 outbreaks lag rainfall, lags, 0 no lag, -1 rainfall lags outbreaks

Lag	Year				
	2005	2006	2007	2008	2009
1	0.046	-0.059	0.228	0.400	0.227
0	-0.090	-0.392	0.047	-0.255	0.018
-1	0.087	-0.186	-0.084	0.056	-0.020

1.4.1.3 Results for Christchurch

Plots of rainfall and Norovirus outbreaks vs. months show a variable annual cycle of rainfall, and no relationship between Norovirus outbreaks and periods of high rainfall (Figure 1.6).

Christchurch shows a slightly different trend in total reported outbreaks for each year. Christchurch has an increase in reported Norovirus outbreaks from 2005 when 12 Norovirus outbreaks were reported to 32 reported Norovirus outbreaks in 2006 and 53 Reported outbreaks in 2007. From 2007 to 2008 there is a significant decrease in reported Norovirus outbreaks (from 53 to 18 respectively). In Christchurch the increase in reported Norovirus outbreaks in 2009 is relatively small (a total of 26 reported outbreaks), and is down on the number of outbreaks reported in 2007 (53 Norovirus outbreaks).

The number of reported Norovirus outbreaks per month is generally relatively small. However in February 2007 ten outbreaks are reported (Figure 1.7). This is interesting as both January and March have much lower reported Norovirus outbreaks (one and two respectively). A similar pattern can be seen in 2009 when October has eight reported Norovirus outbreaks and September has three and November four.

Table 2.6 shows no statistically significant correlations in any year between reported outbreaks and average rainfall.

There is a small lag effect in 2006 rainfall could influence the number of outbreaks in the following month. Similar to that in Wellington in 2008, in Christchurch in 2005 there is a lag effect present for rainfall (Table 1.8), i.e. that the outbreaks in one month is correlated to the rainfall of the following month. This as explained above is not relevant to the current study.

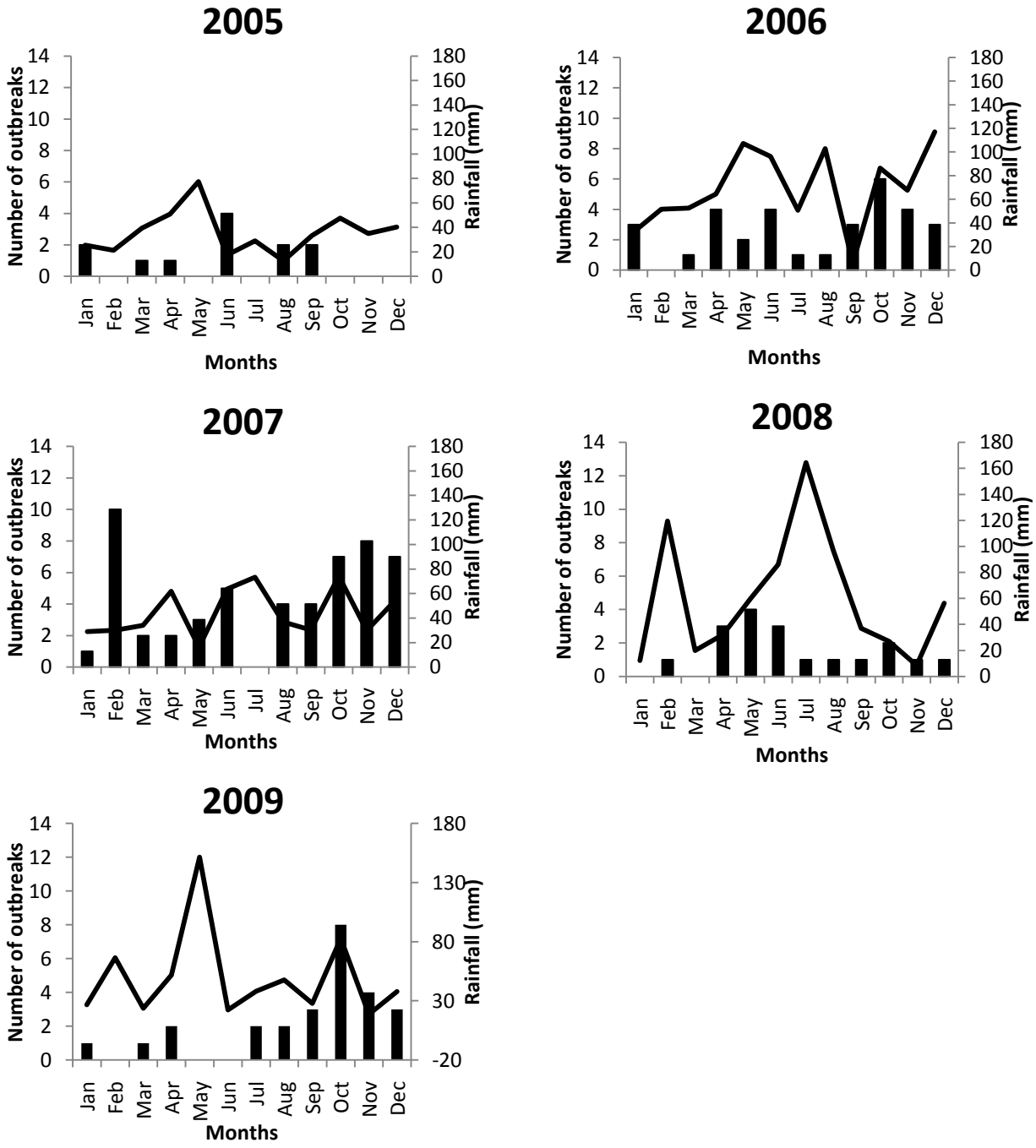

Figure 1.6 Relationship between total monthly recorded Norovirus outbreaks (bars) and average rainfall (line) for Christchurch from 2005 -2009

Table 1.7 Correlation analyses between Norovirus outbreaks and rainfall in Christchurch from 2005-2009

	Year				
	2005	2006	2007	2008	2009
Pearson Correlation	-0.518	0.145	-0.092	0.099	-0.033
P-value	0.084	0.652	0.775	0.759	0.919

Table 1.8 Cross correlation analyses between total monthly recorded Norovirus outbreaks and rainfall in Christchurch from 2005-2009. Lags: 1 outbreaks lag rainfall, lags, 0 no lag, -1 rainfall lags outbreaks

Lag	Year				
	2005	2006	2007	2008	2009
1	0.683	-0.051	-0.268	-0.387	-0.300
0	-0.430	0.145	0.912	0.099	-0.033
-1	0.174	0.267	-0.470	0.251	-0.075

1.4.1.4 Results for Dunedin

Plots of rainfall and Norovirus outbreaks vs. months show an annual cycle of rainfall, but show no relationship between Norovirus outbreaks and periods of high rainfall (Figure 1.7).

The total number of reported Norovirus outbreaks increased for one in 2005 to 12 in 2006 (Figure 2.5). Twelve Norovirus outbreaks were also reported in 2007. There was a small increase to a total of number of Norovirus outbreaks in 2008 to 15. In 2009 a total of 20 Norovirus outbreaks were reported. These numbers of total reported Norovirus outbreaks are well below the numbers reported for Auckland and some of the years in Christchurch.

The number of reported Norovirus outbreaks in Dunedin is noticeable lower than the other three cities. This may be due to Dunedin's relatively small population size (Table 1.1). No relationship is shown between average monthly rainfall and reported Norovirus outbreaks (Figure 2.5).

Table 2.8 shows no significant correlations between outbreaks and rainfall in any of the years studied.

In 2008 Dunedin has a significant lag effect towards rainfall, i.e. that rainfall in one month may influence the number of Norovirus outbreaks in the following month (Table 2.9).

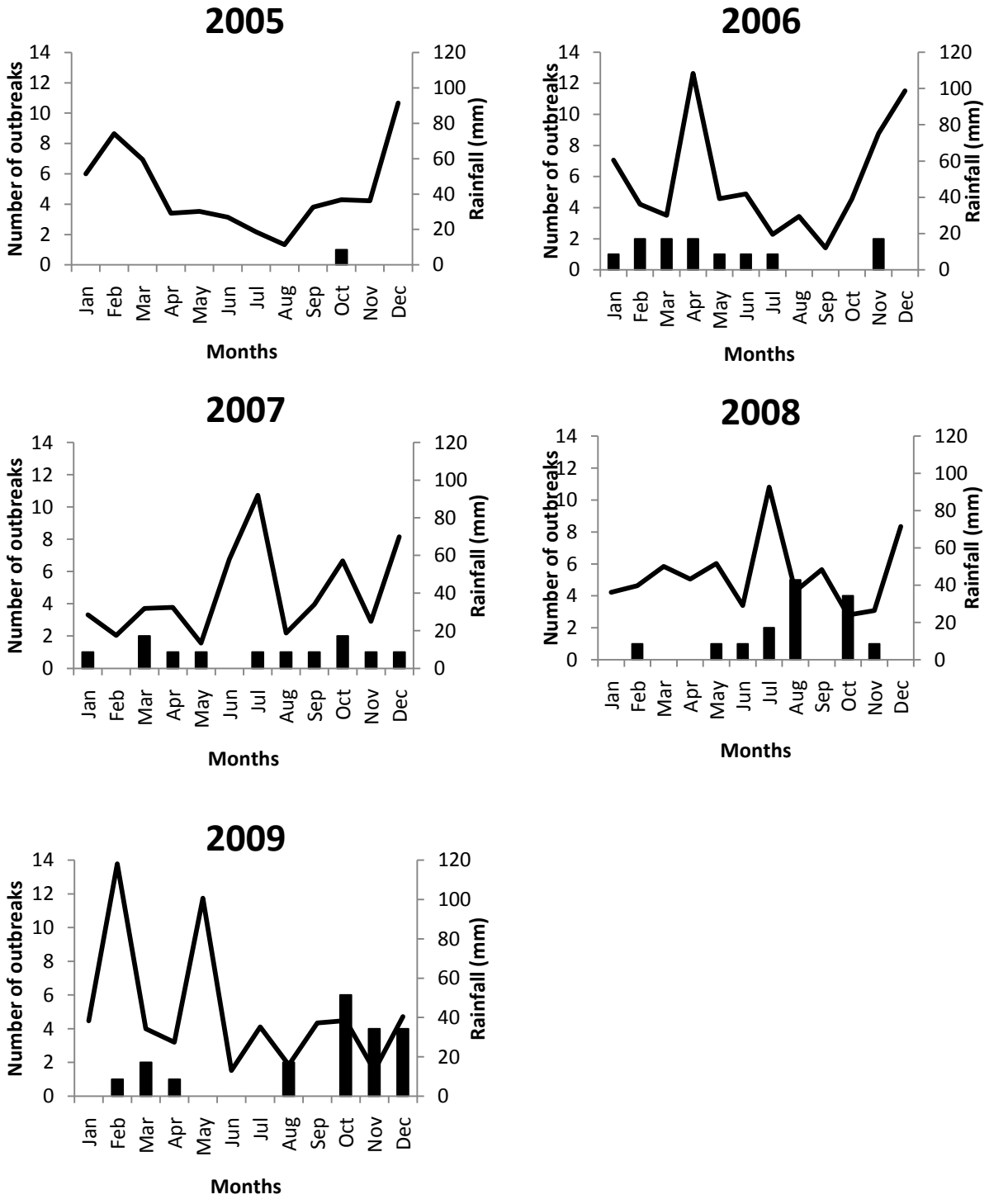

Figure 1.7 Relationship between total monthly recorded Norovirus outbreaks (bars) and average rainfall (line) for Dunedin from 2005 -2009

Table 1.9 Correlation analyses between Norovirus outbreaks and rainfall in Dunedin from 2005-2009

	Year				
	2005	2006	2007	2008	2009
Pearson Correlation	-0.063	0.246	0.085	-0.210	-0.223
P-value	0.846	0.441	0.793	0.512	0.485

Table 1.10 Cross correlation analyses between total monthly recorded Norovirus outbreaks and rainfall in Dunedin from 2005-2009. Lags: 1 outbreaks lag rainfall, lags, 0 no lag, -1 rainfall lags outbreaks.

Lag	Year				
	2005	2006	2007	2008	2009
1	-0.065	0.104	0.059	0.598	-0.067
0	-0.063	0.246	0.085	-0.210	-0.223
-1	-0.061	0.353	-0.415	-0.097	0.255

1.4.1.5 Results for the Nation

Plots of rainfall and Norovirus outbreaks versus. months show a variable annual cycle of rainfall, and no relationship between Norovirus outbreaks and periods of high rainfall (Figure 1.8).

No noticeable relationship between reported Norovirus outbreaks and average rainfall for the National data is apparent (Figure 1.8). In 2006 from March to June there is a decreasing trend in the number of Norovirus outbreaks reported. From June to July the number of Norovirus outbreaks holds and then steadily increases through to October. In 2007 and 2008 there are periods of three months or more when the number of reported Norovirus outbreaks only fluctuates by two or three outbreaks. In 2007 this occurs from April through to August. In 2008 this can be seen from January through to March and from April through to August.

Table 2.10 shows no statistically significant correlations between recorded outbreaks and average rainfall in any year.

In 2006 there is a small significant lag effect for towards rainfall, i.e. that rainfall in may influence the number of Norovirus outbreaks in the following month. In 2009 there is a lag effect towards rainfall, i.e. that outbreaks lag rainfall (Table 1.11). A lag effect is also in effect in 2006 with rainfall lagging reported outbreaks, although it is not relevant in the context of this study.

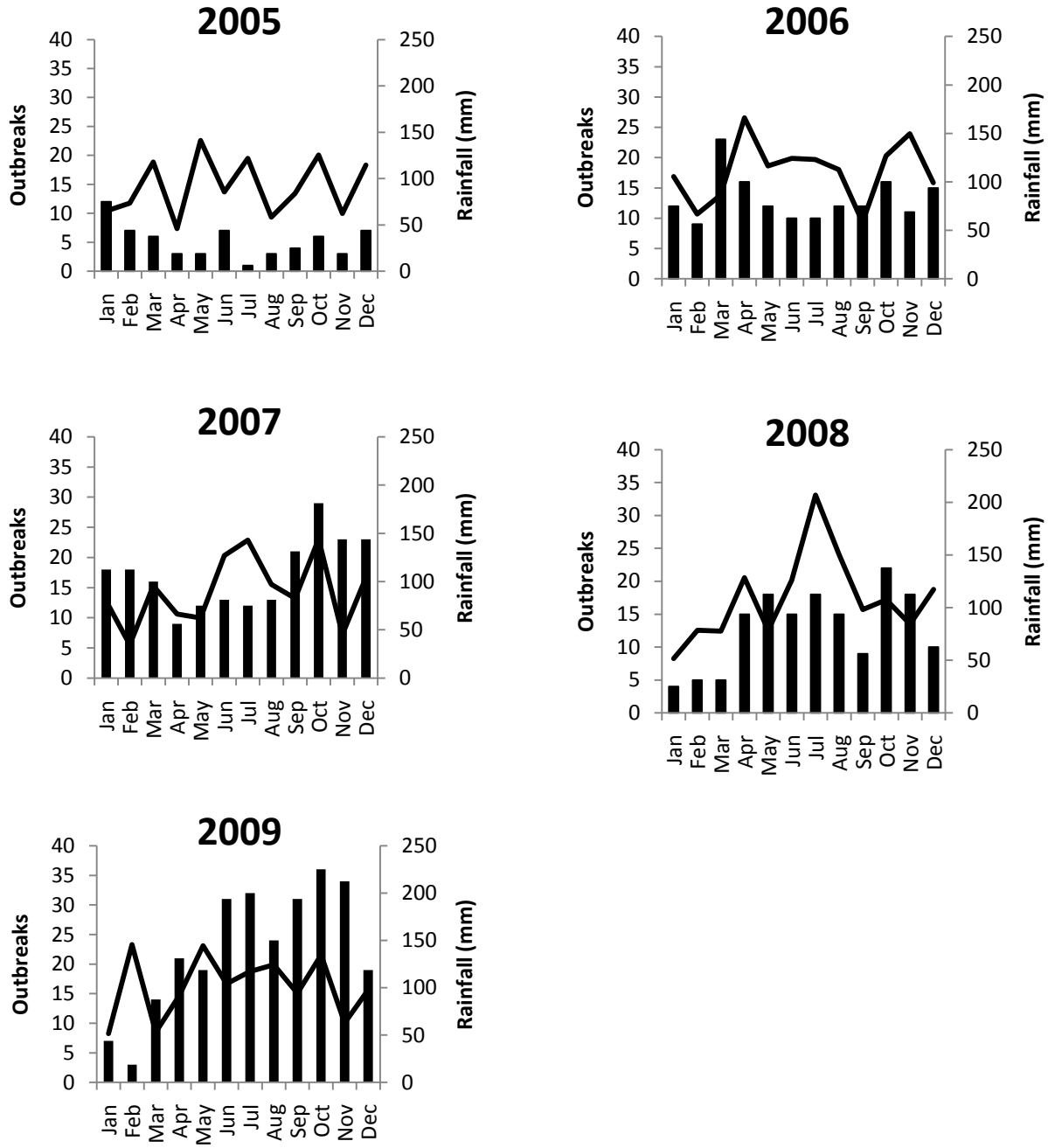

Figure 1.8 Relationship between total monthly recorded Norovirus outbreaks (bars) and average rainfall (line) for the Nation from 2005 -2009

Table 1.11 Correlation analyses between Norovirus outbreaks and rainfall in entire country from 2005-2009

	Year				
	2005	2006	2007	2008	2009
Pearson Correlation	-0.202	0.017	0.108	0.499	0.085
P-value	0.530	0.958	0.737	0.099	0.793

Table 1.12 Cross correlation analyses between total monthly recorded Norovirus outbreaks and rainfall in the entire country from 2005-2009. Lags: 1 outbreaks lag rainfall, lags, 0 no lag, -1 rainfall lags outbreaks

Lag	Year				
	2005	2006	2007	2008	2009
1	0.166	-0.552	-0.078	0.274	0.435
0	-0.201	0.017	-0.108	0.499	0.085
-1	0.036	0.625	-0.192	0.223	0.080

1.4.2 Seasonal Norovirus Outbreaks and Rainfall

For season outbreaks summer is split over two years, for example summer 2006 consists of the monthly data from December 2005 and January and February 2006. Summer 2005 only includes the monthly data for January and February of 2005.

1.4.2.1 Results for Auckland

Plots of rainfall and Norovirus outbreaks vs. season show an annual cycle in rainfall, but no relationship between Norovirus outbreaks and periods of high rainfall (Figure 1.9).

In 2007 an increase in rainfall from autumn to winter and then a steady decline over spring and summer corresponds to an increase in Norovirus outbreaks in winter and spring and then a decrease in summer (Figure 1.9). The high levels in spring may be a result of a lag effect from high winter rainfall. A similar pattern can be found in 2008 without a lag effect.

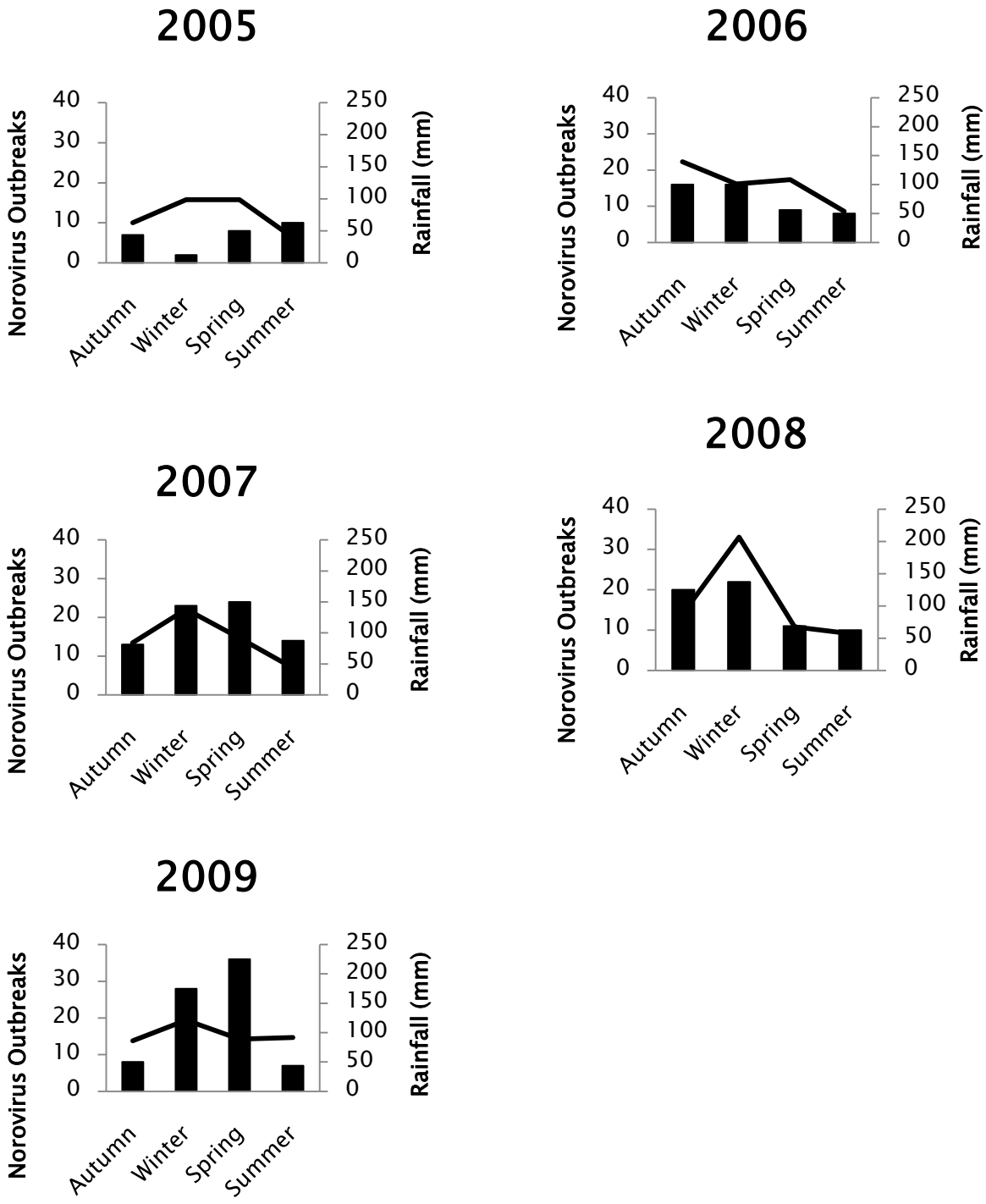

Figure 1.9 Season relationship between Norovirus outbreaks (bars) and rainfall (line) for Auckland from 2005 -2009

Interestingly autumn and winter in 2006 and 2008 have the highest number of outbreaks, while spring and summer have the lowest. In addition the total number of Norovirus outbreaks reported for each pair of season is similar. In 2007 and 2009, winter and spring have the higher number of reported outbreaks and autumn and summer the lowest. It must also be noted that summer for 2009 only contains the number of reported outbreaks for one month, December.

Correlation analysis of total season recorded outbreaks and average season rainfall shows no significant correlation between the two (Table 1.13).

Table 1.13 Correlation analyses between total number of outbreaks recorded in a season and average seasonal rainfall in Auckland

	Year				
	2005	2006	2007	2008	2009
Pearson Correlation	-0.749	0.830	0.447	0.752	0.280
P-value	0.251	0.170	0.553	0.248	0.720

1.4.2.2 Results for Wellington

Plots of rainfall and Norovirus outbreaks vs. season show an annual cycle in rainfall, however there is not relationship between Norovirus outbreaks and periods of high rainfall (Figure 1.10).

No season appears to be constantly dominant in terms of highest number of reported Norovirus outbreaks. Spring in 2005 and 2006 have relatively low outbreak levels, however in 2007 and 2008 spring has the highest number of Norovirus outbreaks of all the seasons, and then in 2009 spring has the second lowest number of reported outbreaks (Figure 1.10).

In 2008 there is an increase in rain from autumn to winter and an increase in outbreaks from winter to spring with a sharp decrease in outbreaks in summer. However in 2009 there is an increase in rainfall from winter to spring and yet the outbreaks decrease from winter to spring quite sharply.

Correlation analysis shows no significant correlations between seasonal outbreaks and average season rainfall (Table 1.14).

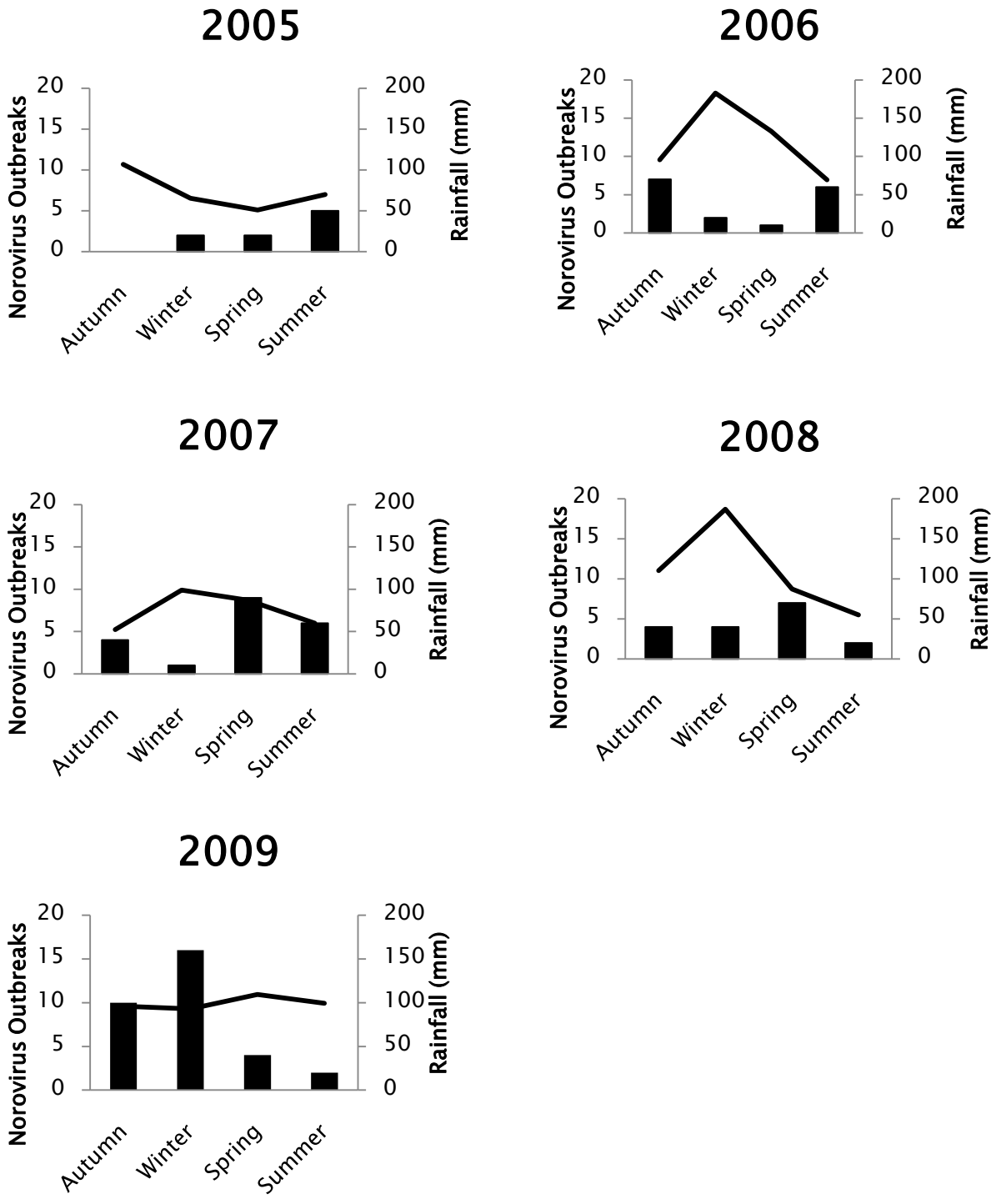

Figure 1.10 Season relationship between Norovirus outbreaks (bars) and rainfall (line) for Wellington from 2005 -2009

Table 1.14 Correlation analyses between total number of outbreaks recorded in a season and average seasonal rainfall in Wellington

	Year				
	2005	2006	2007	2008	2009
Pearson Correlation	-0.0509	-0.780	-0.457	0.033	0.009
P-value	0.491	0.22	0.543	0.967	0.991

1.4.2.3 Results for Christchurch

Plots of rainfall and Norovirus outbreaks vs. season show an annual cycle in rainfall, however there is no relationship between Norovirus outbreaks and periods of high rainfall (Figure 1.11).

Spring had the highest reported number of Norovirus outbreaks in 2006, 2007 and 2009. Winter and summer alternated the second highest number of reported Norovirus outbreaks for these years (Figure 1.11). Spring 2009 had significantly highest number of reported Norovirus outbreaks, than the autumn, winter and summer.

As with Auckland and Wellington correlation analysis shows no significant correlations between seasonal outbreaks and average season rainfall (Table 2.14).

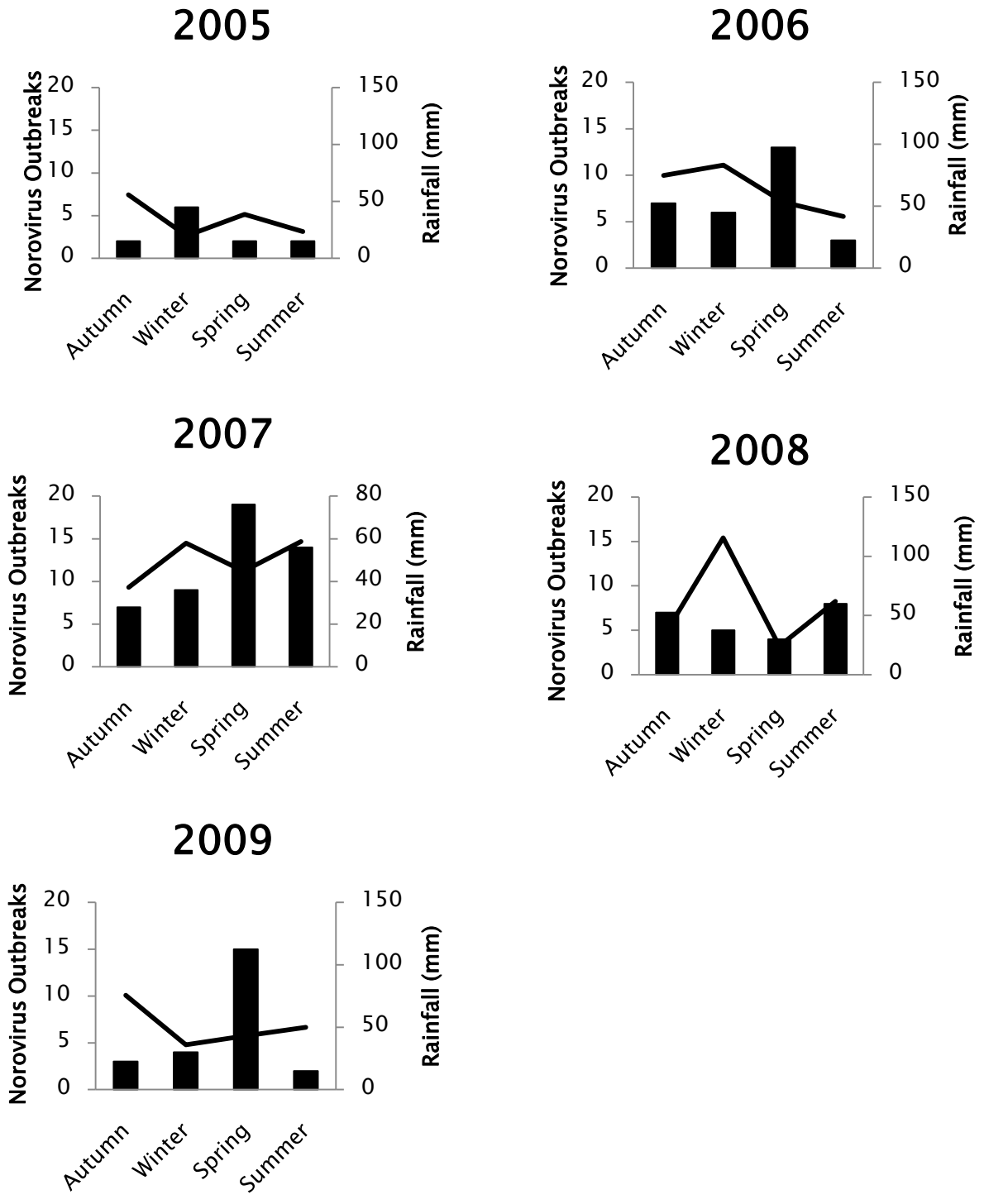

Figure 1.11 Season relationship between Norovirus outbreaks (bars) and rainfall (line) for Christchurch from 2005 -2009

Table 1.15 Correlation analyses between total number of Norovirus outbreaks recorded in a season and average seasonal rainfall in Christchurch

	Year				
	2005	2006	2007	2008	2009
Pearson Correlation	-0.696	-0.853	0.242	-0.079	-0.323
P-value	-.304	0.147	0.758	0.930	0.677

1.4.2.4 Results for Dunedin

Plots of rainfall and Norovirus outbreaks vs. season show an annual cycle in rainfall, there is not relationship between Norovirus outbreaks and periods of high rainfall (Figure 1.9).

Rainfall trend across 2005, 2006 and 2009 are similar (Figure 1.12). Rainfall in 2007 and 2008 has also share their own similar pattern.

In 2006 rain fall decreases from autumn to winter and then a steady increase through to summer with a corresponding trend in reported Norovirus outbreak (Figure 1.12). However this was not statistically significant (Table 1.16). No other similar patterns between average rainfall and reported outbreaks can be seen in any of the other years.

From winter 2008 to summer there is a steady decrease in the number of outbreaks, this decrease continues through autumn and winter of 2009, until a sharp increase in Norovirus outbreaks in spring.

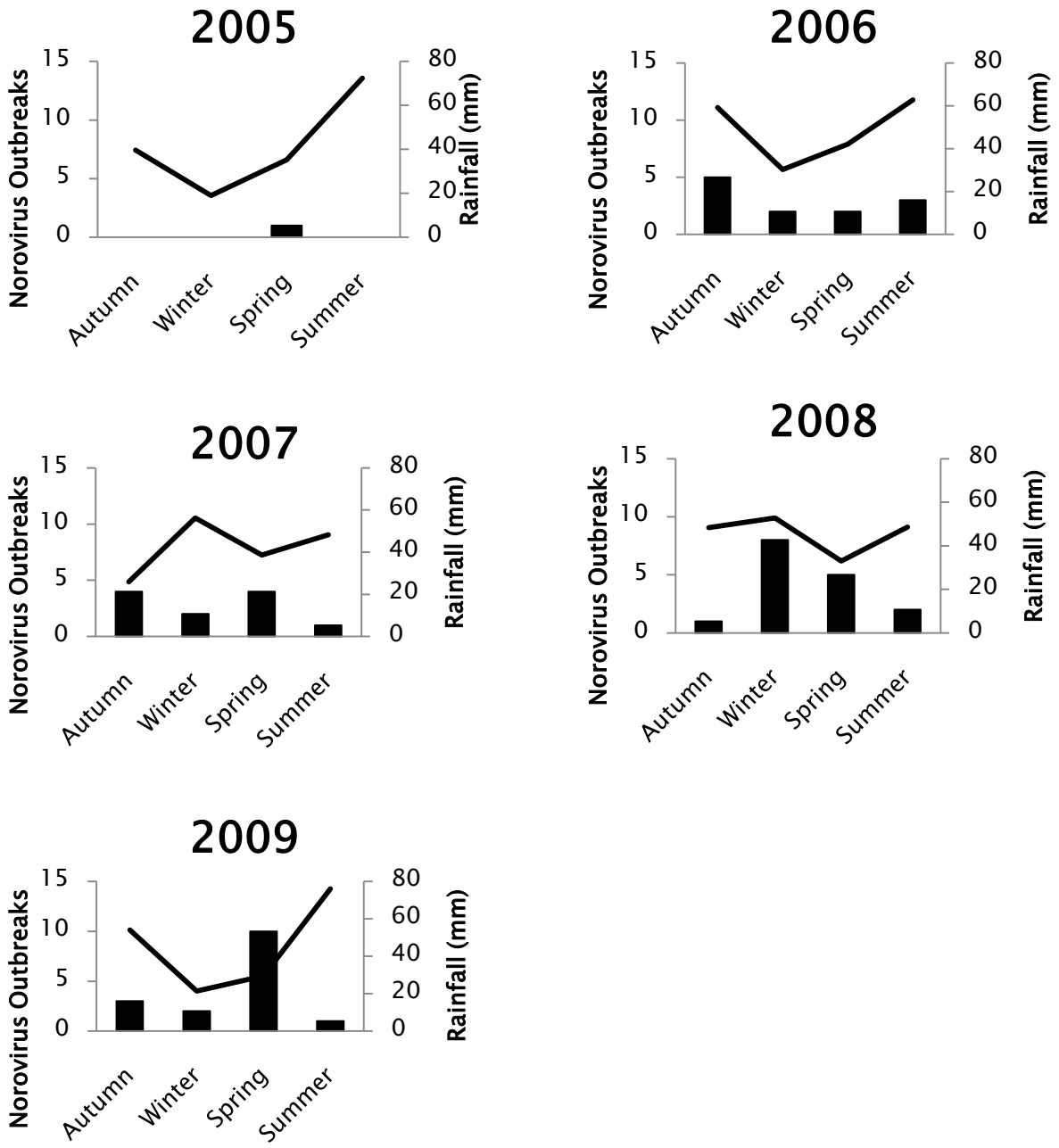

Figure 1.12 Season relationship between Norovirus outbreaks (bars) and rainfall (line) for Dunedin from 2005 -2009

Table 1.16 Correlation analyses between total number of outbreaks recorded in a season and average seasonal rainfall in Dunedin

	Year				
	2005	2006	2007	2008	2009
Pearson Correlation	-0.189	0.680	-0.701	-0.029	-0.122
P-value	0.811	0.320	0.299	0.971	0.888

1.4.2.5 Results for the Nation

Plots of rainfall and Norovirus outbreaks vs. season show an annual cycle in rainfall that changes from 2006 to 2007. However there is no relationship between Norovirus outbreaks and periods of high rainfall (Figure 1.13).

No season is consistently has the highest number of reported Norovirus outbreaks. However spring is generally has one of the higher number of Norovirus outbreaks.

Norovirus outbreaks and rainfall averages in 2005 are relatively sTable across all four seasons. In 2008 there is a large increase in rainfall from autumn to winter however the number of Norovirus outbreaks reported only increases slightly.

There is an increasing trend in the number of reported Norovirus outbreaks from autumn to spring in 2009. Unfortunately the summer data for 2009 only contains the monthly December outbreaks, so there is no way to know if the above increasing trend occurs across the entire year (Figure 1.13).

Table 2.16 shows no significant relationships between recorded outbreaks and rainfall for any of the years studied.

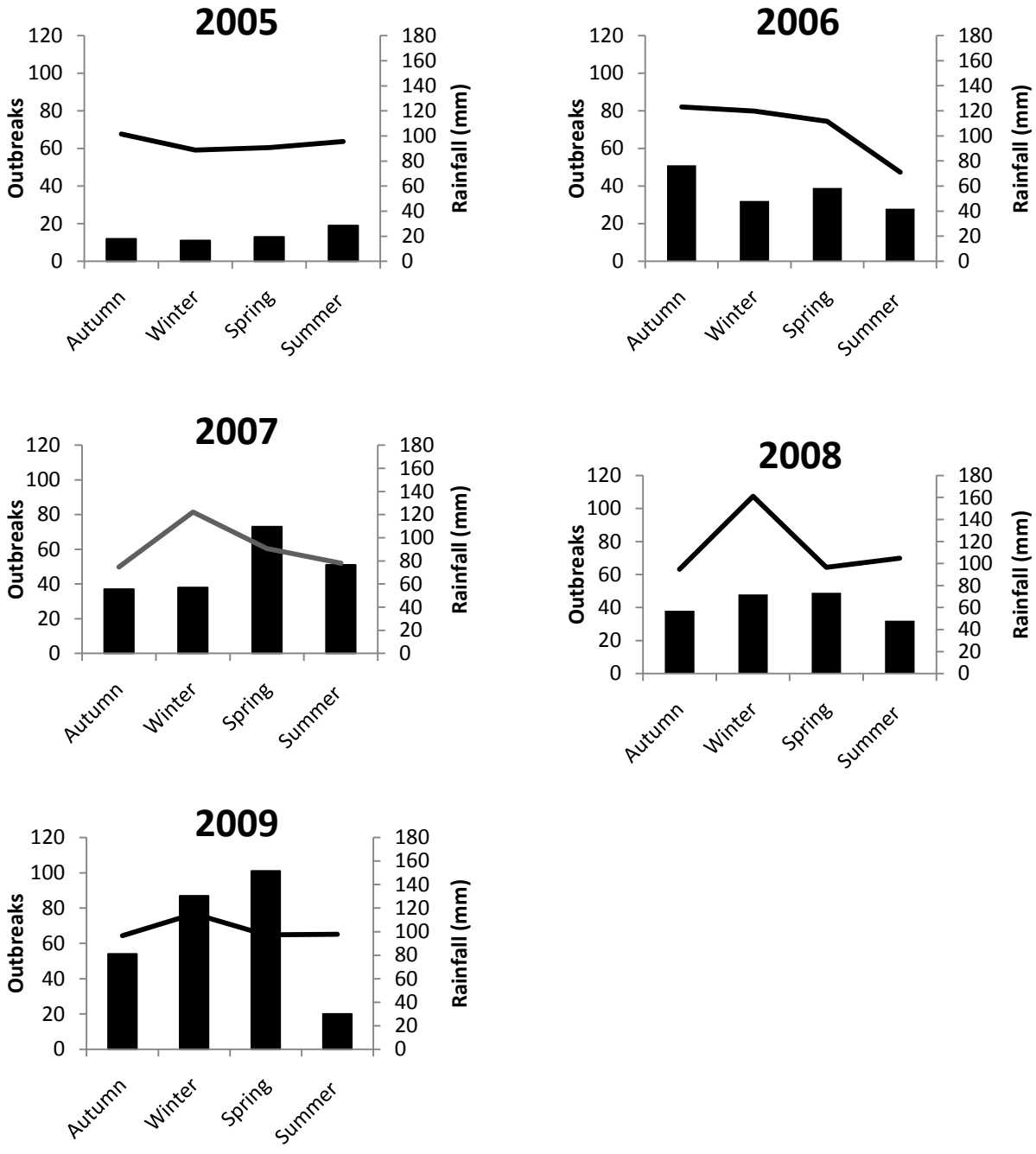

Figure 1.13 Season relationship between Norovirus outbreaks (bars) and rainfall (line) for the Nation from 2005 -2009

Table 1.17 Correlation analyses between total number of outbreaks recorded in a season and average seasonal rainfall for the whole country

	Year				
	2005	2006	2007	2008	2009
Pearson Correlation	0.200	0.677	-0.163	0.429	0.356
P-value	0.800	0.323	0.837	0.571	0.644

1.4.3 Christchurch City Council Environment Data

It is important to note that the levels of contamination found in the following shellfish indicate the presence Norovirus, not the number of viable virions. Using current assay techniques it is impossible to distinguish between viable and non-viable virions.

1.4.3.1 Results for Cockles

Data from cockles collected in the Avon-Heathcote estuary by the Christchurch City Council show no clear relationship between high environmental levels and an increase in Norovirus outbreaks.

Interestingly a high number of Norovirus outbreaks were reported for April, May and June in 2008. After June the number of reported Norovirus outbreaks decreases and remains fairly constant. However in July, September and December of the same year "very high" level of contamination were recorded. Of most interest is the "very high" level reported in July, directly after the period of increased outbreaks in the community (Figure 1.14). Table 1.18 shows the levels of Norovirus virions present for each classification level.

There were no reported Norovirus outbreaks in May and June of 2009. In cockle samples from June and September 'high' levels of viral contamination was recorded. There is an increase in Norovirus outbreaks from July till a peak in October of 2009, and then a subsequent drop in reported outbreaks in November and December.

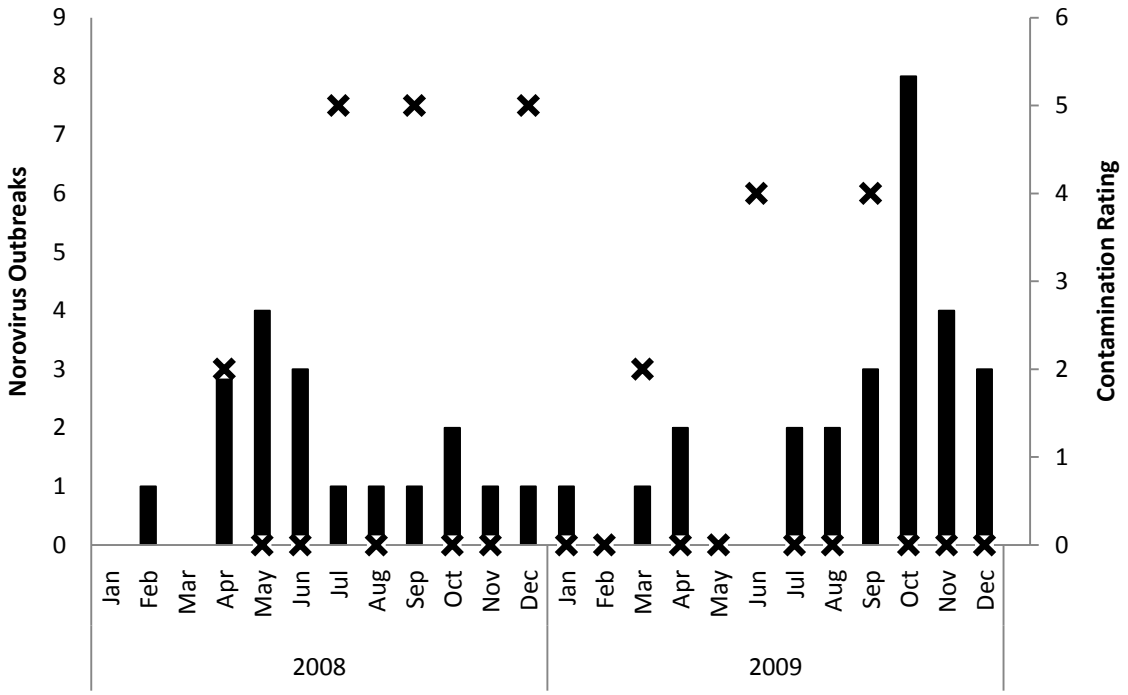

Figure 1.14 Norovirus outbreaks (bars) and recorded contamination in cockles(X) in the Avon-Heathcote Estuary in Christchurch.. Cockle contamination is rated on a scale; 0-no data, 1-not detected, 2-low, 3-moderate, 4-high, 5- very high, 6-extremely high.

Table1.18 Classification of Norovirus virions present for each relative level

Relative levels	
Low	Detectable and to 1.9 log RTPCRU/gram shellfish guts
Moderate	1.9 - 2.2 log RTPCRU/gram shellfish guts
High	>2.2 - 3 log RTPCRU/gram shellfish guts
Very High	>3 - 4 log RTPCRU/gram shellfish guts
Extremely high	> 4 log RTPCRU/gram shellfish guts

1.4.3.2 Results for Tuatua

Norovirus was present in 16 out of the 19 samples (Figure 1.15). Similar results to that of the cockles.

Interestingly no Norovirus outbreaks were reported in January 2008 and no Norovirus virions were present in the tuatua sampled. In March 2008 Norovirus was found to be present in tuatua but there were no recorded incidences for Norovirus that month. Other than March, every month sampled where tuatua were contaminated, Norovirus outbreaks were reported in the Christchurch community

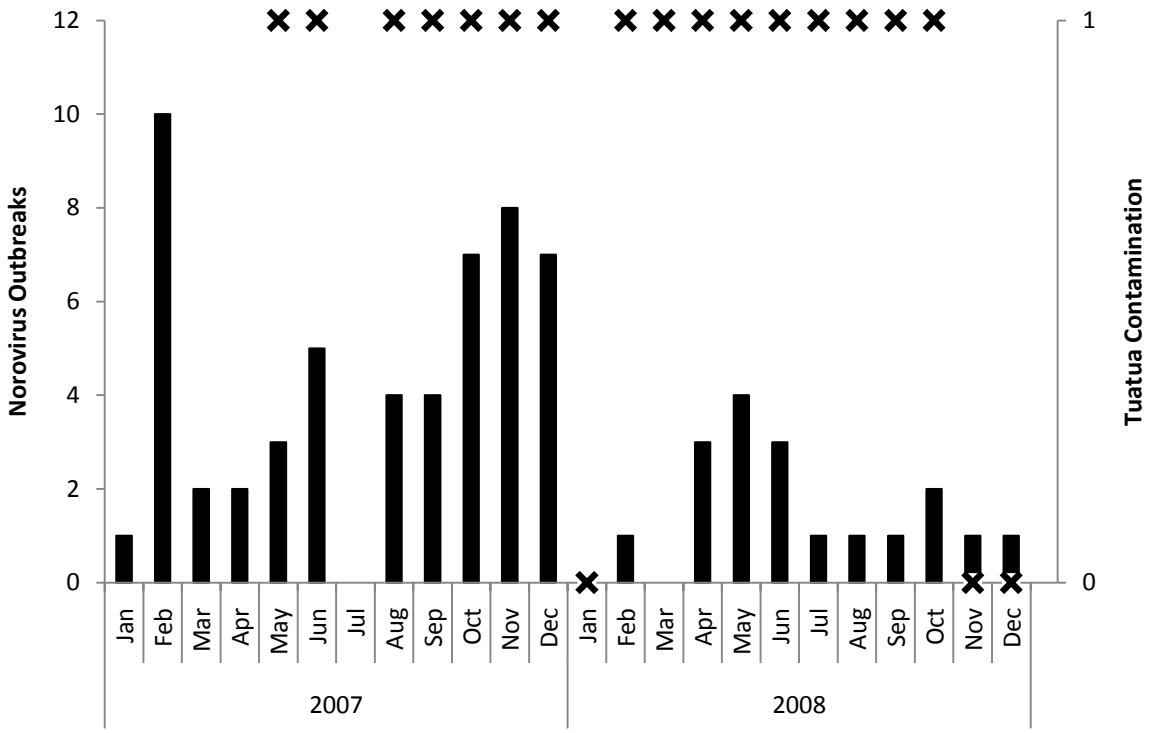

Figure1.15 Norovirus outbreaks (bars) and recorded contamination in tuatua (X) in the Avon-Heathcote Estuary in Christchurch. Tuatua contamination is recorded as present 1, or absent 0,of Norovirus virions. Months with no cross were not sampled.

1.5: Discussion

With the exception of one statistically significant result in Auckland in 2008, no significant relationships were observed between rainfall and reported Norovirus outbreaks, in a monthly or season setting. This result is interesting as it is well documented that Norovirus is present in treated sewage (Shieh *et al*., 2003; van den Berg *et al*., 2005), and that heavy rainfall is known to cause overflows at treatment plants that lead to untreated or partial treated sewage being released into the environment (Wellington City Council, 2010a).

A relationship between population size and the number of Norovirus outbreaks reported is clearly demonstrated in this study. Auckland with a population of over 1.3 million people has significantly more reported Norovirus outbreaks than Dunedin with a population of less than 200,000. Increased population sizes, like that of Auckland, generally allows for more distinct and clear trends to be apparent. With only a few outbreaks each month, as seen in Dunedin, it is difficult to imply that environmental factors such as rainfall are having an impact on the

prevalence of outbreaks of Norovirus. However, when looking at results from Auckland, Wellington, Christchurch and nation figures, partial trends can be identified, although these trends were proven to be not statistically significant.

The monthly and regional scale of this investigation could be too large to portray an effect between rainfall and reported Norovirus outbreaks. A consequence of using average rainfall over a large region could allow for the effects of certain area or period of high rainfall to be minimised or lost. It is difficult to confirm heavy rainfall and sewage overflows or leaks with an outbreak of a virus. It requires a detailed spill reports and sampling at the correct time period and location (Greening and Lewis, 2007).

The effect of heavy rainfall and subsequent sewage overflow may not be immediately noticed. In commercial farming of shellfish, harvesting after heavy rainfall is generally postponed for a week or so to allow the animals to self cleanse themselves. Alternatively if they are harvested they may be placed in tanks or moved to a 'clean' area of water for the purposes of depuration. However, as mentioned earlier Norovirus has been found to persist in shellfish for several weeks after contamination, with depuration methods proven ineffective at the removal of virions.

In a non-commercial farming setting, shellfish could be consumed after heavy rainfall periods. In addition the persistence of Norovirus in shellfish many influence the number of reported Norovirus outbreaks in the following month, but not significantly longer. For these reasons a lag period in the effect of rainfall on Norovirus outbreaks was investigated. Overall where there was a lag effect from rainfall (as shown by -1 on cross-correlation tables) it was small (the closer to 1 the stronger the correlation is considered). The strongest lag observed was in 2006 for the national results (cross-correlation = 0.625).

An important factor to consider is the majority of reported Norovirus outbreaks come from hospital and rest home settings. Due to the set up of these facilities, incidences of Norovirus are likely to be noticed and reported and infect a large number of people this explains the reason why the number of outbreaks rather than the number of cases in an outbreak was investigated in this study.

It is possible that there a number of days, potentially weeks between when a Norovirus outbreak is reported to when it actually occurred. This could explain the weakness of the relationship between Norovirus outbreaks and Rainfall.

It is important to note that only reported outbreaks of Norovirus are used in this study. As mentioned above many of these are in hospitals and rest homes. Due to the short duration of symptoms, usually less than 72 hours (Donaldson *et al*., 2008), many people are unlikely to visit the doctor. This may be especially true if they believe the cause of their illness is due to something they ate. Norovirus is a non-notifiable illness therefore even if a person was to visit their doctor; the doctor has no obligation to test for Norovirus or report cases. They may do so if they are aware of an outbreak in the region but are very unlikely to for a one off case of an upset stomach. This could help explain for the lack of seasonality of Norovirus outbreaks observed.

In the northern hemisphere, Norovirus has been reported as being a winter illness, commonly referred to as "winter vomiting" (Cowden, 2002; Patel *et al*., 2009). However in recent years Norovirus outbreaks have been responsible for an increase in outbreaks in summer across Europe (da Silva *et al*., 2007). The New Zealand Food Safety Authority (NZFSA) suggests that Norovirus outbreaks in New Zealand are more prominent in summer and autumn (New Zealand Food Safety Authority, 2010). Contrastingly, and in agreement with Greening *et al* (2009) this study found no prominent season for Norovirus outbreaks in New Zealand. Additional explanations to the lack of obvious seasonality of Norovirus outbreaks in New Zealand (other than a lack of reporting mentioned above) could include the persistence of the virus in the shellfish and the continued shedding of virions for up to two weeks after symptoms have gone.

The NZFSAs' suggestion that a higher proportion of outbreaks should occur in the summer and autumn months is not without merit. During the summer months many people are on vacation and while the weather is warm one might expect an increase in wild shellfish collecting. Over these months boating is also very popular, especially around the Northland–Coromandel area. The Northland-Coromandel area is also an important shellfish aquaculture region, especially for Pacific oysters (*Crassostrea gigas*) and Greenshell mussels (*Perna canaliculus*). After a number of Norovirus outbreaks oyster from this region the Northland Regional Council set in place to up graded the sewage system in the region and implemented strict regulations for the

disposal of sewage on board boats. While this is generally hard to enforce as one has to be 'caught in the act' of disposing effluent, in January 2009 a yachtie was prosecuted for the disposing of human effluent over the side of his vessel, in the Opua Basin (Northland Regional Council, 2009). It is hoped that measures like this and upgrades of land based sewage treatment will decrease the likelihood of Norovirus contamination in shellfish from sewage incidences in this area (Northland Regional Council, 2002; Gee, 2006; Northland Regional Council, 2006; Northland Regional Council; 2007, Northland Regional Council, 2009).

Norovirus outbreaks in the Christchurch community were plotted against levels of Norovirus contamination found in tuatua and cockles in the Christchurch region (Figures 1.14 and 1.15). As mentioned earlier these shellfish were collected as part of a resource consent process for the construction of a new sewage outfall pipe. With the exception of March 2008, Norovirus outbreaks coincided with periods when the presence of Norovirus was confirmed in tuatua. Interestingly in January 2008, no Norovirus outbreaks were reported and Norovirus was not detected in tuatua for the same month. This may be coincidental as Norovirus is known to persist in the environment for up to two months (Greening *et al*., 2003).

In addition, only one outbreak was reported in February 2009 and no outbreaks were reported in March, although cockles tested positive for Norovirus virions during this period. Whether the presence of Norovirus virions in tuatua and cockles are good indicators of Norovirus outbreaks in the community or not, it is hoped that the construction of a new sewage outfall pipe in Christchurch is hoped to decrease the levels of faecal contamination and enteric pathogens observed in tuatua and cockles in the area. When at maximum capacity the pipe which was officially opened in late March 2010, can carry 6 m^3/s of treated wastewater from the treatment plant to be discharged 3 km out into Pegasus Bay (Christchurch City Council, 2010). It will be interesting to see of a decrease in enteric pathogen contamination such as Norovirus in shellfish occurs and if this results in fewer Norovirus outbreaks in the community.

Shellfish gathered from the wild are not under the same strict quality control as commercially farmed shellfish. Many regional councils in New Zealand have in place water and shellfish monitoring programmes. When quality levels are breached, the appropriate signs and warnings are posted. However, many of the water monitoring programmes only monitor bacteriological standards. It has been demonstrated in numerous studies that bacteriological standards may not be adequate indicators of viral contamination (Shieh *et al*., 2003; Greening and Lewis, 2007).

Greening and Lewis (2007) investigated the prevalence of enteric viruses and bacteriophage in shellfish at a number of different sites around New Zealand. The aim of the study was to look at the relationship between the occurrence of F-RNA phage (a potential indicator of faceal and viral contamination) and enteric viruses in shellfish, and to determine whether local shellfish were contaminated with human enteric viruses from sewage. Oysters, pipi, cockles and mussels were collected monthly or bimonthly. Sites sampled included shellfish harvesting areas and areas downstream of sewage outfall. The sites were located in Dunedin, Napier, Kaipara, Kerikeri, the Bay of Islands and Whangaroa. Forty-eight percent of the shellfish collected tested positive for one or more human enteric viruses. F-RNA phage was detected in 211/318 shellfish samples, but their presence was not clearly associated with the presences of viruses, except in an area where shellfish were growing in close proximity to a sewage outfall.

In a commercial setting shellfish are unlikely to be farmed near a sewage outfall. Therefore in areas not associated with sewage disposal, bacteriological quality may not be a reliable indicator for the presence of viruses. Indeed when bacteriological standards are breached it could be conferred that human enteric viruses, such as Norovirus may be present in high concentrations. While not all areas are monitored, it is likely that if aware of the location of sewage outfall pipes those individuals will avoid collecting shellfish from those areas.

Gathering of shellfish from the wild is an important custom to many Māori in New Zealand. The collection of wild shellfish is associated with a higher risk factor than shellfish purchased through a supermarket. People who become ill after the consumption of wild shellfish are generally less likely to visit a medical professional, such as a doctor if they believe it was from shellfish. Indeed it is these cases that go unreported, and may substantially lower the number of Norovirus outbreaks reported.

In conclusion this study finds that reported Norovirus outbreaks do not correlate with rainfall in the major cities of New Zealand.

Chapter 2: Cadmium Exposure in New Zealand

2.1 Introduction to Cadmium

Cadmium is a heavy metal that occurs naturally at low concentrations in the Earth's crust. Cadmium is also present at low concentrations in the air, soils and oceans worldwide. Most commonly associated with the ores of zinc, lead and copper (Satarug *et al*., 2003), cadmium is an extremely versatile metal. Used in the construction of sleeve bearings for automotive, aircraft and marine engines, in pigments and plastics stabilizers. Cadmium can also be found in numerous household items from utensils, phosphors in television tubes, household appliances, transistors, batteries, and hardware fittings such as nuts and bolts and screws (Flick *et al*., 1971).

This versatile metal is considered non-essential for human health and increased exposure to cadmium is responsible for a wide range of human health concerns. Cadmium has been classified as a type 1 human carcinogen. In addition the effects of increased intake can included reproductive toxicity (Mahalik *et al*., 1995), neurotoxicity (Méndez-Armenta and Ríos, 2007), an immunosuppressive effects (Blakley, 1985). Occupation exposure and smoking are the major contributors to increased cadmium intake in humans (Järup L, 2002; Satarug *et al*., 2003; Järup and Åkesson, 2009). For people who are not occupationally exposed to cadmium and do not smoke, dietary intake is the main source of exposure.

2.1.1 Cadmium in Air

Ambient cadmium concentrations in air comprises both natural and anthropogenic sources. Cadmium's primary form in air is a mixture of oxide, chloride and sulphate compounds (WHO Regional Office for Europe, 2000). Natural emissions account for around 10-15 % of cadmium levels in air and can originate from volcanic activity, forest fires, and erosion and weathering of cadmium containing rock. The remaining 85-90% is produced by anthropogenic sources, mainly from smelting and refining of non-ferrous metals, fossil fuel combustion and municipal waste incineration (WHO Regional Office for Europe, 2000). In Canada, 78% of atmospheric cadmium originates from the copper and nickel industries and 17% from the combustion of fossil fuels (Crete *et al*., 1989). Atmospheric cadmium concentrations are generally highest around areas in which cadmium is emitted by industry, for example zinc smelters. This can cause health concerns for people that work and live around these areas as inhaled cadmium is the most efficiently absorbed form of cadmium.

2.1.2 Cadmium in Soils

Cadmium occurs naturally in soils, this concentration can be increased through anthropogenic activities. Mining and smelting operations of zinc, lead and copper ores are some of the major contributors to increased soils cadmium levels (Pigeot *et al*., 2006; Bradley and Cox, 1986; Little and Martin, 1972). Agricultural and horticultural soils are impacted by increased cadmium through the application of phosphate fertilisers and sewage sludge (Järup, 2002; de Meeus *et al*., 2002; Satarug *et al*., 2003).

Once in soils, cadmium can become mobile and subsequently available for uptake by plants and animals (de Meeus *et al*., 2002). A number of factors influence the mobility of cadmium in soil. Two important factors are the acidity and organic matter content (Olsson *et al*., 2002). Mobile cadmium can be readily absorbed by vegetation or lost to the air or aquatic environments (Alloway *et al*., 1990; Arnold *et al*., 2006; Das and Jana, 2003; Forstner and Ravera, 1984; Roberts *et al*., 1994; Williams and Harrison, 1984). Certain plants more readily accumulate cadmium, e.g. potatoes and cereals such as rice, than others (Alloway *et al*., 1990). Animals which graze on vegetation grown on high cadmium soils have an increased risk of accumulating cadmium in their bodies (Loganathan and Hedley, 1997). Cadmium is primarily stored in the liver and kidneys of mammals, consequently the consumption of these organs by humans can increased cadmium intake and body burden.

New Zealand is a largely agricultural country, known for its sheep, beef and dairy production. In order to sustain pastures and increase crop growth, large amounts of fertilisers are applied. The concern is that approximately 80% of cadmium applied to soils in phosphate (P) fertiliser remains in the topsoil (Taylor, 1997). Superphosphate fertilisers applied in New Zealand, and a number of other countries including Australia and the United Kingdom, were historically produced from cadmium-rich phosphate rock originating from Nauru (a small island nation in the South Pacific Ocean). Nauru phosphate rocks are now known to contain some of the highest cadmium content found in phosphate rocks, averaging 450 mg Cd/kg P (Taylor, 1997; Satarug *et al*., 2003; Taylor *et al*., 2007; Cadmium Working Group, 2008). In recent decades New Zealand fertiliser manufactures have used phosphate rocks with significantly lower cadmium contents, from China, Morocco and Togo with a cadmium content of between 10-340 mg Cd/kg P. The New Zealand fertiliser industry also introduced a voluntary limit for cadmium concentrations in fertilisers, 280 mg Cd/ kg P (Cadmium Working Group, 2008). New Zealand

soils due to their volcanic origins are also generally very acidic (Cadmium Working Group, 2008), which can increase mobility of cadmium in soils.

Irrigation of pastures can increase the levels of cadmium leaches from soil (Butler and Timperley, 1996). Similar to other parts of the world, flood irrigation has traditionally been the most practiced form of irrigation in New Zealand (McDowell, 2009). Generally, this involves irrigation waters travelling along a race until gates allow the water to flood a bay. The bays gently slope downwards to allow water to reach the opposite end. The application of too much water can allow for valuable soil and nutrients are lost from the bays, into streams and rivers. In recent decades there has been a move away from flood irrigation into more efficient systems, including guns, K-line, centre-pivots and drip and sprinkler systems. These systems allow for better control over the amount of water and area to be irrigated.

2.1.3 Cadmium in Water

Elevated cadmium concentrations in aquatic environments can occur as a result of numerous anthropogenic activities; disposal of mining tails, industrial and municipal wastes, and runoff from urban land. In freshwater cadmium occurs in three forms; Cd^{2+}, $Cd(OH)_2$ and $CdCO_3$ (Ravera, 1984). Cadmium has a low affinity for organic ligands but a high affinity for the chloride ion. Consequently, organic complexes appear to be restricted to the lower salinity reaches of estuaries (Ravera, 1984). Increasing salinity is accompanied by an increase in the amount of various chlorocomplexes and a reduction in the activity of the free ions. In pure seawater cadmium exists almost entirely as chloride species ($CdCl^+$, $CdCl_2$, and $CdCl_3^-$), with a small amount of Cd^{2+} (Ray, 1984; Turner *et al*., 2008).

Natural concentrations of cadmium in oceans vary between oceans and with vertical profiles. In addition, cadmium is strongly correlated with phosphate and nitrate concentrations in the subsurface waters of the ocean. In the oceanic waters surrounding Foveaux Strait in New Zealand, cadmium has a bilinear relationship with phosphate, with a pronounced kink (Frew and Hunter, 1995). The kink is attributed to the influence of low cadmium waters from Antarctic Intermediate waters and to the formation of high cadmium Antarctic bottom waters near Antarctica (Frew and Hunter, 1995). Cadmium concentrations in coastal waters are heavily influence by cadmium inputs from the land, and can be elevated in areas tidal flushing is poor, e.g. enclosed bays and estuaries (Hunter and Ho, 1991).

2.1.4 Cadmium in Shellfish

There are two main pathways for cadmium accumulation in shellfish; directly as dissolved cadmium through their gills or indirectly through diet. Bioavailable particulate cadmium occurs in a number of forms; adsorbed to largely inorganic sediment, complexed to detrital organic matter or contain in living algae (Ray, 1984). Wild shellfish occur in various environments: buried in sediments (e.g. cockles (*A. strutchburyi)* and tuatua (*P. subtriangulata)*), in intertidal areas (oysters (*C. glomerata)* and mussels (*Perna canaliculus)*) and on the ocean floor (oysters (*O. lutaria)* and scallops (*Pecten novaezelandiae)*). Commercial aquaculture farms are generally located in enclosed bays. These areas have low suspended sediment concentrations but high nutrient concentrations and algal productivity.

As mentioned in chapter 1oysters have a large filtration capacity that allows for rapid accumulation and concentration of substances from the surrounding waters. Cheng and Gobas (2007) investigated filtration rates in the oyster (*C. virginica).* In the presence of phytoplankton, oysters feed continuously and remained open on average of 94% of the time (Cheng and Gobas, 2007). When placed in a tank without plankton they only remained open on average 35% of the time. Shellfish aquaculture methods generally involve shellfish living continuously submerged and therefore feeding, 24 hours a day. This constant feeding could contribute to the high levels of cadmium found in some cultured oysters (Cheng and Gobas, 2007).

High cadmium concentrations in shellfish have the potential to become a significant management problem for shellfish aquaculture. Several countries have import limits for cadmium concentrations in shellfish. In 2000 British Columbian oysters were rejected in Hong Kong for exceeding the import limit of 2 parts per million (ppm) (Kruzynski, 2004, Cheng and Gobas, 2007). The New Zealand dredge oyster (*Ostrea lutaria),* commonly referred to as Bluff oysters) also contain high levels of cadmium, with concentrations as high as 7.9 µg/g wet weight reported (Nielsen, 1975). The exact cause of the high concentrations of cadmium is this oyster is not known. The area they grow in is limited in anthropogenic sources of cadmium. Frew *et al* (1996) proposed that the cadmium depleted waters in the area are due to an unusually high uptake by phytoplankton. Possibly due to a deficiency in another essential trace element, such as zinc. This increased cadmium uptake by phytoplankton would lead to a cycle of cadmium-enriched biogenic particulate matter that consequently would result in cadmium enrichment in bottom sediments and benthic filter feeders, and might explain high cadmium levels in Bluff oysters.

2.1.5 Human Exposure to Cadmium

Human exposure to cadmium is generally limited to three routes: occupational exposure, cigarette smoking and dietary intake. Dietary intake is the most common route of exposure for non-smokers and non-occupationally exposed individuals. Cadmium is absorbed by the body through inhalation and absorption in the gut. Approximately 20-50% of inhaled cadmium is absorbed and only approximately 5% of ingested cadmium. Absorption of cadmium in the gut can increase to at least 20% in the presence of calcium- and/or iron-deficiencies (Friberg, 1984; Olsson *et al*., 2002). Cadmium accumulation occurs over a lifetime, body burdens at birth are virtually zero. Accumulated cadmium is primarily stored in the liver and kidneys. Cadmium burdens increases in the body due to its long biological half-life of approximately 10-30 years (Friberg, 1984; Järup, 2002).

Occupational exposure to cadmium can occur from zinc smelters, pigment and battery factories and through soldering activities. The most significant contemporary source of occupational exposure results from the production of cadmium-nickel batteries. Approximately 55% of cadmium production is used in the production of these batteries (WHO Regional Office for Europe, 2000). Excessive long-term inhalation of cadmium can result in severe pulmonary oedema and chemical pneumonitis leading to respiratory failure and death (Järup, 2002).

Individuals who smoke cigarettes are known to have an increased cadmium body burden. This cadmium intake is from bioaccumulated cadmium in the tobacco plant, and highly absorbable cadmium oxide is formed during the burning of cigarettes. Approximately 50% of inhaled cadmium is absorbed by smokers (Satarug *et al*., 2003). Cadmium concentrations in cigarettes vary with type and brand, generally one cigarette contains roughly 1-2 µg cadmium (Elinder *et al*., 1983; Järup and Åkesson, 2009).

Dietary intake of cadmium is highly variable indeed some individuals and populations are at risk of higher cadmium exposure than others. Relatively high concentrations of cadmium are present in molluscs and crustaceans, such as oysters and other bivalves, cephalopods and crabs (especially in brown meat). As cadmium is mainly stored in the liver and kidneys of mammals the consumption of these organs increases dietary cadmium intake. As mentioned previously cadmium is accumulated over a life time, therefore older an animal the higher the cadmium concentration. This becomes important in populations where animals such as horse, mink and moose are consumed (Liu, 2003; Gamberg *et al*., 2005; Arnold *et al*., 2006). These animals are

generally slaughtered at an older age than sheep and cattle. In a study on mink in Canada, cadmium concentration was found to be positively correlated to age ($p = 0.009$) (Gamberg *et al*., 2005). In a study by Arnold *et al* (2006) cadmium concentrations in the kidney cortex of Alaskan moose (*Alces alces*) were found to vary with different populations. The lowest median was record in the Yakutat population (1.39µg/g, range = 0.47-3.96 µg/g) and the highest median was recorded in Galena (19.7 µg/g, range = 9.49-65.7 µg/g).

Cadmium is present in a wide variety of other foods: oil seeds, cocoa beans and in certain wild mushrooms. Cereals such as rice and wheat, green leafy vegetables, potatoes and root vegetables such as carrots often contain higher concentrations of cadmium than other plant foods. Meat, eggs, milk and dairy products are generally low in cadmium (Vannoort and Thomson, 2005; Järup and Åkesson, 2009). Dietary intake of food and drink containing cadmium concentrations in excess of approximately 15mg/g can give rise to acute gastrointestinal symptoms including vomiting, diarrhea and abdominal cramps. Adverse effects on the kidneys as a result of low-level long-term exposure to cadmium are typically considered to be the critical health effects in humans (Järup, 2002).

The Joint Food and Agriculture Organisation and World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA) established in its 16th meeting a Provisional Tolerable Weekly Intake for cadmium for 400 to 500 µg of cadmium per adult person, this equates to 0.8 - 1.01 µg/kg/day for a 70 kg person (Ministry of the Environment, 2010). Many regulatory agencies worldwide have provided evidence for reconsideration on the weekly tolerable intake amount for cadmium, with concerns over the small safety margins in the current standard. For example the European Food Safety Authority's Panel on contaminants in the food chain set a reduced tolerable weekly intake for cadmium of 2.5 µg/kg bw (European Food Safety Authority, 2009). The JECFA has indicated that cadmium is likely to be reviewed as part of its 73rd meeting in June 2010 (Ministry of the Environment, 2010).

2.1.6 Cadmium Distribution in the Body

Cadmium found in the kidney is generally the result of long-term low level exposure (Friberg, 1984). Approximately one-third of the cadmium in the body can be found in the kidneys. Cadmium concentrations in the liver are the result of recent cadmium exposure. Conversely, accumulation of cadmium in the kidneys is the result of long term exposure (Järup, 2002).

Cadmium flows in blood to the kidney where cadmium bound to metallothionein is filtered with the primary urine and reabsorbed into the tubular cells, is the same as with other low molecular weight proteins (Friberg, 1984). The reabsorption of cadmium metallothionein is almost complete at low levels of cadmium in plasma however; the reabsorption may be less effective at high levels of metallothionein in plasma. A continuous catabolism of cadmium metallothionein takes place after reabsorption in the tubuli, cadmium is split from the metallothionein and bound to newly formed metallothionein in the tubular cells. Kidney damage is prevented until a stage is reached at which the kidney can no longer produce enough metallothionein to bind to levels of cadmium present (Friberg, 1984). It is at this stage the non-metallothionein-bound cadmium ions become very toxic. Some of the cadmium filtered through the glomeruli and some of the stored cadmium in the kidney is excreted via the urine.

Urinary cadmium concentration is a good indicator of cellular levels and an indicator of kidney burden. Cadmium in urine can therefore be useful as an indicator of cadmium burden in the body (Friberg, 1984; Järup, 2002). The amount of excreted daily is very small and only approximated 0.005-0.015 % of the total body burden, which corresponds to its long biological half life (WHO Regional Office for Europe, 2000, European Food Safety Authority, 2009). Urinary cadmium increases dramatically when kidney damage occurs. Cadmium levels in the kidney cortex are approximately 2.25 times higher than in the kidney as a whole. Concentrations in the renal cortex increase with age until the age of 50-60 years, after which it levels off or even decreases (Lyon *et al*., 1999; WHO Regional Office for Europe, 2000). The critical concentration of cadmium in the renal cortex is approximately 200 mg/kg at these levels numerous detrimental effects can occur; tubular or mixed type proteinuria, aminoaciduria, glucosuria and hypercalciuria and morphologically, and lesions predominantly involving the tubules (Friberg, 1984; Nogawa *et al*., 1986; WHO Regional Office for Europe, 2000).

Cadmium is well known for its detrimental effects on the kidney (Friberg, 1984). In addition to its effects on the kidney, cadmium is known to cause numerous other detrimental effects; nephrotoxicity, osteteoporosis, teratogenic, neurotoxicity, genotoxicity and endocrine ad reproductive effects (Mahalik *et al*., 1995; Méndez-Armenta and Ríos, 2007; European Food Safety Authority, 2009). It is known carcinogen, studies as early as 1956 have shown that rats when exposed to a single subcutaneous injection of cadmium chloride develop irreversible testicular damage (Flick *et al*., 1971). In Japan increased cadmium intake from the consumption of contaminated rice caused the disease, Itai-itai. Itai-itai is a combination of severe kidney

damage and osteomalacia. It occurred among inhabitants in certain areas of Toyama prefecture in Japan, the rice became heavily contaminated due to irrigation of the soil with contaminated water form a mine in the mountains (Friberg, 1984; Nordberg, 2009).

2.1.7 Cadmium and the Immune System

The influence of cadmium on the immune system is controversial, and there is much uncertainty around the mechanisms of cadmium induced immunotoxicity.

The most conflicting results are in studies on the humoral immune responses of rodents. This is because cadmium has been shown to enhance (Ohsawa *et al*., 1988), suppresse (Blakley, 1985) and exert no influence (Blakley, 1988) on the immune system. However, in cell mediated responses, phagocytosis and host resistance assays results consistently show a depression in the immune response (Simonet *et al*., 1984). Ohsawa *et al* (1988) reported no change in B and T lymphocyte count following oral cadmium administration, while sub-cutaneous injection resulted in major reduction in B cells with no change in T lymphocytes. *In vivo* exposure to cadmium caused thymic damage, resulting in a significant decrease in thymic weight, T-cell depletion, thymic atrophy and splenomegaly.

Data available on humans is limited. In a study on cadmium workers by Karakaya *et al* (1994), there was no significant difference in serum IgG, IgM and IgA concentrations and a control group. Ritz *et al* (1998) investigated the impaired immunity in school children in a heavily cadmium polluted regions of Germany. The study found increasing body burden of cadmium was associated consistently with dose-dependent suppression of immediate hypersensitivity and of IgG but not IgM, IgA or IgE. They concluded that cadmium impaired the secondary humoral response only (Ritz *et al*., 1998). Despite this significant conjecture there is clear evidence for an effect of cadmium in the immune response.

2.2 Objectives

To study:

1) Cadmium levels in soils, oceans, selected shellfish, dietary intake and body burden in New Zealand and compare them with levels in Canada, Italy and the United Kingdom.

2) Levels at which cadmium can impair the immune system

2.3 Methodology

2.3.1 Literature review/Meta-analysis

A comprehensive search of several databases and search engines was conducted to find data on cadmium levels in soils, oceans, shellfish (oysters and mussels), concentrations in human kidneys, dietary intakes and immunosuppressive levels in animals, principally mice.

The databases used included:

Web of Science®
Google Scholar®
Proquest®
Science Direct®
Springerlink®
Wiley Interscience®

The searches were conducted with a combination of the following key words:

Cadmium,
Fertilizers,
Soils,
Oceans,
Shellfish,
Oysters,
Mussels,
Humans,
Kidney, and
Immune system.

Research articles were also identified from reference lists and bibliographies of articles found during electronic searches. Only articles in English were used.

Electronic searches started October 2008 and concluded July 2010.

The meta-analysis is based on results published in peer-review journals and that from other agencies however this may not represent all available information as other relevant articles may be published in another language, format or may not have been published at all.

2.3.2Meta-analysis

2.3.2.1 Investigated Countries

Data from New Zealand, Canada, Italy and the United Kingdom (UK) were studied. These countries were chosen to compare and evaluate environmental and dietary cadmium concentrations in New Zealand.

Canada was chosen due to high levels of cadmium found in British Columbian oysters. In addition Canada is one of the world's largest producers of Cadmium.

Italy has is a volcanic country, with numerous active volcanoes. Offal is also popular in Italy and moreover horse. Indeed offal is known to be rich in cadmium. In addition Italy coastal geography allows for plentiful supply and consumption of shellfish.

British soils are generally naturally low in minerals, in addition there is little modern day mining of metals. For these reasons the United Kingdom is considered a low cadmium environment.

2.3.2.2 No Observable Effect Level (NOEL)

An estimate of the immunosuppressive No Observable Effect Level (NOEL) of cadmium was calculated from published studies in mice following *ad libitum* exposure to cadmium in drinking water. The approximate cadmium dose received by the mice was calculated using published values for daily water consumption in mice.

2.3.3 Conversion of Units

The various data sources had varying scales/units. These have all been converted to a common scale/unit for the purposes of this study. The meta-analysis was calculated using the following computational equations.

2.3.3.1 Soil

Convert µg/g to mg/kg no conversion necessary
Convert ppm to mg/kg no conversion necessary

2.3.3.2 Oceans

To covert nmol/kg to nmol/l:
nmol/kg/density of seawater
Density of seawater = 1.025 g/ml
= nmol/kg / 1.025

To convert µg/l to nmol/l:
Cadmium atomic weight = 112.4
To convert mol to nmol:
112.4/1000 = 0.1124 nmol

To convert µg/l to nmol/l:
µg/l / 0.1124

2.3.3.3Kidney

To convert dry weight to wet weight:
Dry weight/water content supplied in articles

2.3.3.4 Average Cadmium Intake

To convert µg/kg body weight/week to mg/day:
µg/kg body weight/week

To convert µg to mg:
µg/ 1000
mg/kg body weight/week
multiply by body weight supplied
mg/week
divide by 7 to get mg/day

2.3.3.5 No Observable Effect Level

Calculation given in results section

2.4: Results Cadmium Exposure

2.4.1 Cadmium Concentration in Soil

Soil cadmium concentrations are variable with depth; indeed surface soils are more heavily impacted by environmental contaminants. For this reason where possible, when investigating soil cadmium levels samples in the top 10-15cm have been used.

Soil cadmium concentration varied considerably between the four countries (Table 2.1, Figure 2.1). Rural Italy has the lowest mean (0.22 mg/kg). With the exception of the Hamps and Manifold in the UK, the means concentrations for cadmium in soil are less than 1.5 mg/kg.
New Zealand has one of the lowest mean soil cadmium concentrations.

Canada (1.1 mg/kg) has a higher mean soil cadmium concentration than New Zealand (0.35 mg/kg), and the three Italian sites (Table 2.1). The ranges for both Manitoba and Port Colborne fall within the range of New Zealand (Figure 2.1).

Mean soil cadmium concentration for all three Italian sites vary. In addition as the mean increases so does the concentrations of the ranges. Italy has some of the smallest means and ranges of the four countries (Figure 2.1).

Soil cadmium concentrations in the UK comprise both the largest ranges and the highest means. Interestingly the range of cadmium concentrations in the Hamps is relatively small when compared to the range observed in the Manifold (which extends far beyond the scale of Figure 2.1 to 21.858 mg/kg), they have reasonably similar mean soil cadmium concentrations (2.14 and 2.47 mg/kg respectively) (Table 2.1).

Table 2.1 Concentrations of cadmium present in soil (mg/kg) and the soil depth at which the sample was taken from New Zealand, Canada, Italy and the UK

Country	Cadmium Concentration (mg/kg)	Soil sample depth	Source
New Zealand	Mean = 0.35 Range = 0-2.52	0-10cm	Taylor *et al*., 2007
Canada Port Colborne Schools and beaches	Range = 0.2 – 1.0	0-5cm	McIlveen, 2000
Canada Thompson Manitoba	Mean = 1.1 Range = 0.4-2.5	0-2 cm	Jones and Phillips, 2003
Italy Rural	Mean=0.22 Range=0.08-0.60	0-10cm	Polemio *et al*., 1982
Italy Industrial	Mean=0.50 Range=0.10-1.20	0-10 cm	Polemio *et al*., 1982
Italy Urban	Median = 0.68 Range = 0.27-1.86	0-10cm	Manta *et al*., 2002
UK Hamps	Mean=2.14 Range= 0.39-6.57		Bradley and Cox, 1986
UK Manifold	Mean=2.47 Range=0.254-21.858		Bradley and Cox, 1986
UK Yorkshire	Mean = 1.4 Median =1.2 Range = 0.3–3.8	0-10-15cm	Akbar *et al*., 2006

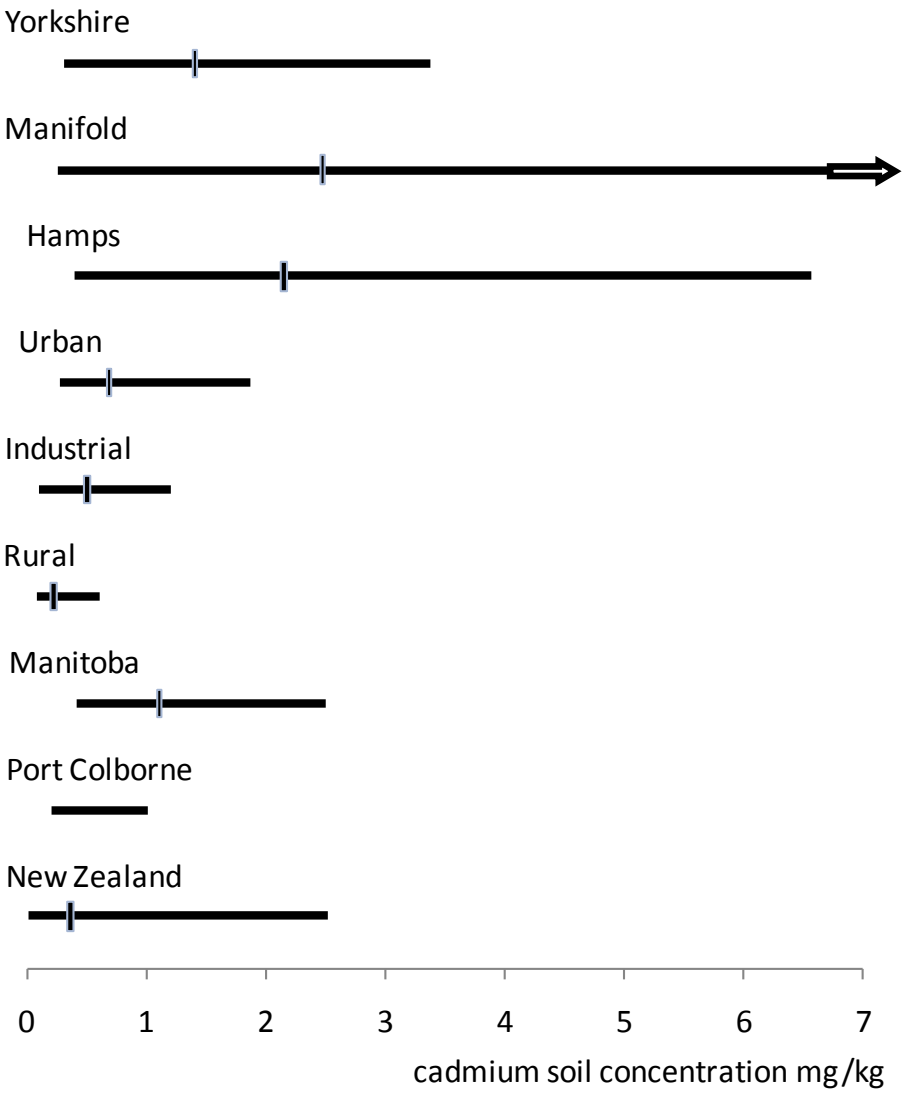

Figure 2.1 Bar graph depicting the ranges and means (vertical line) of the various cadmium soil concentrations. Note that the cadmium concentration for Manifold extends further than the horizontal axis.

2.4.2 Cadmium Concentrations in Oceans

Cadmium concentrations vary with vertical profile in the ocean. Therefore only results from the top 50 m are considered in this meta-analysis. Cadmium concentrations in the ocean range between 0-2.0 nmol/l (Table 2.2, Figure 2.2). The largest range comes from the various estuarine samples in the UK.

The range of oceanic cadmium concentrations in New Zealand is similar to that of the UK (Figure 2.2). The mean cadmium concentration observed for Puyseger Trench is similar to that observed of the various UK estuarine areas, 0.36 and 0.436 nmol/l, respectively (Table 2.2). Cadmium concentrations for the Challenger, Regina leg, and Otago Peninsula are all within the concentration range seen in Foveaux Strait. In addition they are lower than the cadmium concentration mean in Puyseger Trench.

Deep Bay in Canada has the second highest upper range for cadmium concentrations observed in oceans (0.74 nmol/l). Only the various estuarine areas in England have a higher concentration range (1.96 nmol/l). Lemmens Bay has a range which falls into the mid to high concentrations of cadmium (Figure 2.2).

The Italian samples are among some of the lower concentrations of cadmium observed (Table 2.2). The samples from the Southern and Northern Adriatic seas are similar in concentration, and when compared to the means seen in the other three countries they are the lowest, and fall into the lower observed ranges.

Table 2.2 Cadmium levels in the surface waters of oceans around New Zealand, Canada, Italy and the UK

Country	Cadmium concentration nmol/l Dissolved	Depth	Source
New Zealand Puyseger Trench	Mean = 0.36[a]	Surface	Frew and Hunter, 1995
New Zealand Foveaux Strait	Range = 0.02-0.58[b]	15-50 m	Frew and Hunter, 1995
New Zealand Otago Peninsula	Range = 0.088 – 0.214[c]	Surface	Croot and Hunter, 1998
New Zealand Reinga leg	Range = 0.02 – 0.12[d]	0.1-25m	Hunter and Ho, 1991
New Zealand Challenger	Range = 0.17-0.22[e]	15-25m	Hunter and Ho, 1991
Canada Vancouver Island, Deep Bay	Range = 0.44-0.79[f]	5m	Lekhi *et al*., 2008
Canada Vancouver Island, Lemmens Inlet	Range = 0.20-0.45[g]	5m	Lekhi *et al*., 2008
Italy Northern Adriatic Sea	Mean = 0.081[h]		Tankere and Statham, 1996
Italy Southern Adriatic Sea	Mean = 0.074[i]	0-50m	Tankere and Statham, 1996
England North Sea	Range = 0.036-0.454[j]	Surface (<5m)	Laslett, 1995
England English Channel	Range = 0.098-0.196[k]	Surface (<5m)	Laslett, 1995
England Irish Sea	Range = 0.116-0.721[l]	Surface (<5m)	Laslett, 1995
England Various near shore/estuarine	mean = 0.436[m] Range= 0.08-1.96[n]	Surface	Laslett, 1995

[a] converted nmol/kg to nmol/l
0.37/1.025 (density of seawater) = 0.36 nmol/l
[b] converted nmol/kg to nmol/l
0.02/1.025=0.02
0.59/1.025=0.58
[C] converted nmol/kg to nmol/l
0.090/1.025=0.088
0.219/1.025=0.214

[d] converted nmol/kg to nmol/l
0.02/1.025=0.02
0.12/1.025=0.12
[e] converted nmol/kg to nmol/l
0.17/1.025=0.17
0.23/1.025=0.22
[f] converted nmol to nmol/l
0.45/1.025=0.44
0.81/1.025=0.79
[g] converted nmol to nmol/l
0.21/1.025=0.20
0.46/1.025=0.45
[h] converted nmol to nmol/l
0.083/1.025=0.081
[i] converted nmol to nmol/l
0.076/1.025=0.074
[j] convert µg/l to nmol/l
0.004/0.1124 (Cd atomic weight)=0.036
0.051/0.1124=0.454
[k] convert µg/l to nmol/l
0.011/0.1124=0.098
0.022/0.1124=0.196
[l] convert µg/l to nmol/l
0.013/0.1124=0.116
0.081/0.1124=0.721
[m] convert µg/l to nmol/l
Mean: 0.049/0/1124=0.436
[n] convert µg/l to nmol/l
0.009/0.1124=0.08
0.220/0.1124=1.96

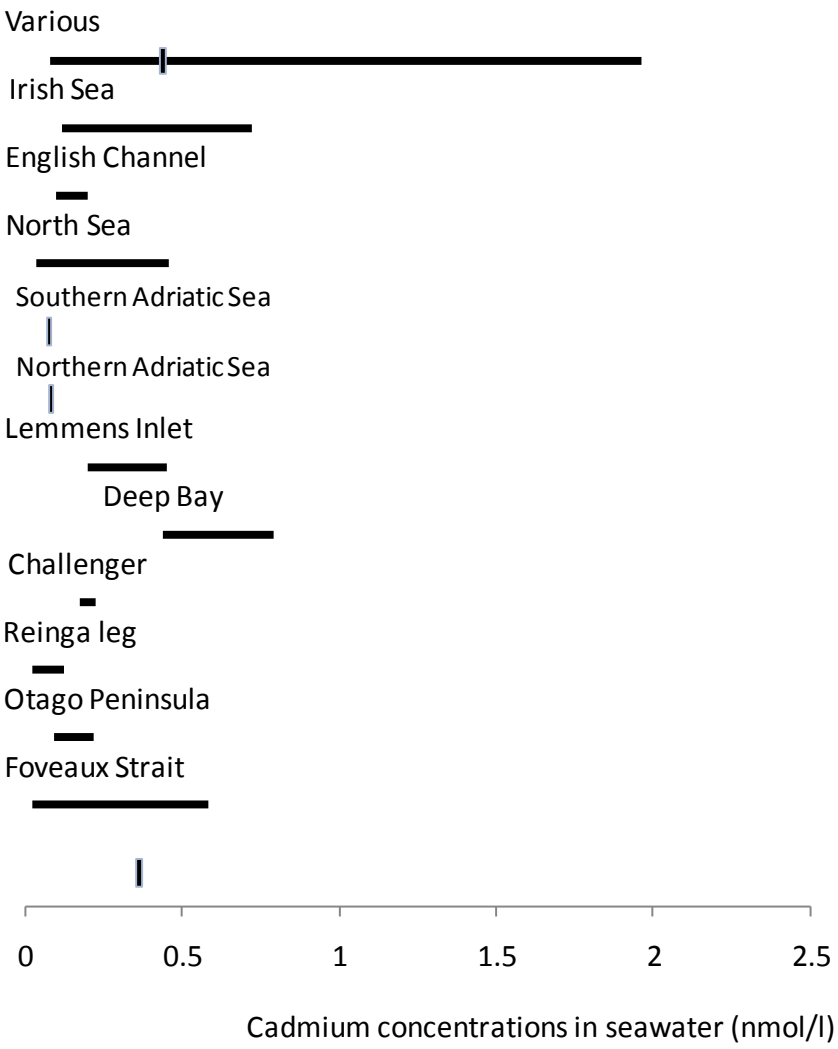

Figure 2.2 Bar graph depicting the ranges and means (vertical line) of the various cadmium seawater concentrations (nmol/l)

2.4.3Cadmium Concentrations in Oysters

Cadmium concentrations in oysters show quite clearly that New Zealand has a relatively large range (Table 2.3, Figure 2.3). The ranges observed in Canada are within those of New Zealand.

The species *Ostrea lutaria* from in New Zealand has the largest range and highest upper ranges of cadmium concentrations observed (Table 2.3). The stomach content data for Mahurangi catchment was excluded from Figure 3.3 as all the other examples where of whole body weight. Interestingly the cadmium concentration observed in the stomach content of the oysters from Mahurangi catchment is much larger (2.1 µg/g) than that of the mean body weight of animals found in the same catchment (Table 2.3). This indicates that majority of cadmium is stored in the gut of the animal.

Canadian data from Deep Bay and Lemmens Inlet are within the low to mid range of cadmium levels found in the New Zealand oyster, *O. lutaria* (Figure 2.3). Interestingly the species *C. gigas* observed in Canada has similar concentration range to the same species observed in New Zealand (Table 2.3).

The range observed for Italy is slightly above that of the UK, and at the lower concentrations of cadmium observed for Canada (Figure 2.3). In relation to New Zealand, it is within the lower limits of the North Island ranges (Bay of Islands, Great Barrier and Mahurangi catchment), but below the ranges of the South Island samples.

The UK has significantly lower mean cadmium concentrations and ranges compare to Canada and New Zealand (Table 2.3, Figure 2.3). The UK data also demonstrated the difference in cadmium concentration pre and post spawning. Indeed the mean cadmium concentration is higher in pre spawning.

Table 2.3 Cadmium concentrations present in various oyster species from New Zealand, Canada, Italy and UK

Country	Cadmium Concentration (µg/g wet weight)	Species	Source
New Zealand Foveaux Strait	3.4-7.9	*O. lutaria*	Nielsen and Nathan, 1975
New Zealand Stewart Island	1.4 – 7.1	*O. lutaria*	Nielsen and Nathan, 1975
New Zealand Tasman Bay	1.9-5.9	*O. lutaria*	Nielsen and Nathan, 1975
New Zealand Bay of Islands	0.60 – 2.0	*C. glomerata*	Nielsen and Nathan, 1975
New Zealand Great Barrier	0.71 – 3.8	*C. glomerata*	Nielsen and Nathan, 1975
New Zealand Mahurangi catchment	Range = 0.17-0.33	*C. gigas* whole body	Butler and Timperley, 1996
New Zealand Mahurangi catchment	Mean = 2.1	*C. gigas* stomach content	Butler and Timperley, 1996
Canada Vancouver Island Deep Bay	Range = 1.17 - 3.57	Cultured *C. gigas*	Lekhi *et al*., 2008
Canada Vancouver Island Lemmens Inlet	Range = 1.40 – 2.47	Cultured *C. gigas*	Lekhi *et al*., 2008
Canada Vancouver Island Deep Bay	Range = 2.38– 3.02	Wild *C. gigas*	Widmeyer and Bendell-Young, 2008
Italy Adriatic Sea	Range = 0.63-1.18	*Ostrea edulis*	Martincic *et al*., 1987
UK	Mean=0.21		Portmann, 1979
UK	Mean = 0.296 Range = 0.105-0.682	*O. edulis*	Food Standards Agency, 2005
UK	Mean = 0.132 Range = 0.69-0.204	*C. gigas* (post spawning)	Food Standards Agency, 2005
UK	Mean = 0.242 Range = 0.095-0.461	*C. gigas* (pre spawning)	Food Standards Agency, 2005

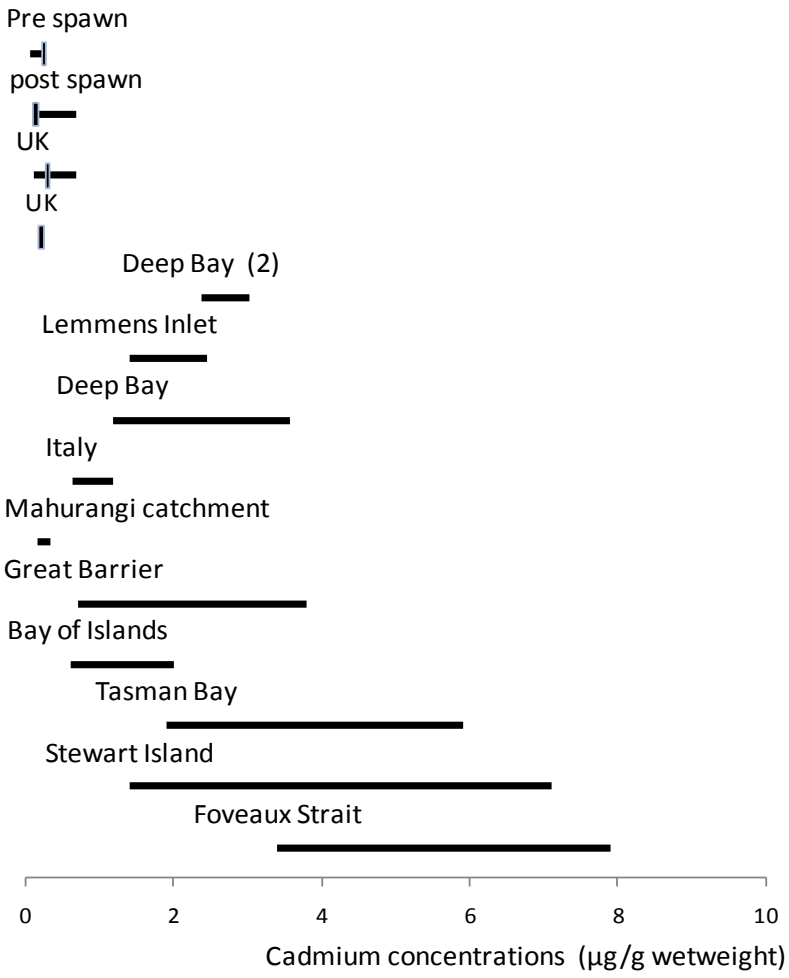

Figure 2.3 Bar graph depicting the ranges and means (vertical line) of the various cadmium concentrations in oysters (µg/g wet weight)

2.4.4 Cadmium Concentration in Mussels

Cadmium concentrations observed in mussels are generally lower than those found in oysters. The exceptions to this are mussels from the Gulf of Gaeta and Adriatic Sea in Italy (Table 2.4, Figure 2.4).

Unlike the cadmium concentrations found in oysters in New Zealand, the concentration of cadmium in mussels is some of the lowest observed (Table 2.4). The lowest concentrations can be observed from Waitangi Bridge and Opuna (0.07 and 0.11 µg/g respectively). Cadmium concentrations found in mussels from Urapukapuka are in the middle of the overall concentration range (Figure 2.4).

The four measurements from Barkley Sounds in Canada had different means. Overall the levels observed from mussels in this area fall in the mid concentrations of cadmium observed (Figure 2.4).

The mean cadmium concentration and range observed in the UK lies within the New Zealand results and at the bottom end of the Canadian results.

Cadmium concentrations observed in Gaeta and the Adriatic Sea (2001), Italy, are well above the means concentrations and ranges of New Zealand, Canada and the UK (Figure 2.4).Interestingly the mussels from the Adriatic Sea in 1987 are in line with concentrations observed in mussels in the other three countries.

Table 2.4 Cadmium concentrations present in various mussel species from New Zealand, Canada, Italy and the UK

Country	Cadmium Concentration (µg/g wet weight)	Species	Source
New Zealand Bay of Islands, Urapukapuka	Mean = 0.75	*Perna canaliculus*	Whyte *et al*., 2009
New Zealand Bay of Islands, Opua Wharf	Mean = 0.11	*P. canaliculus*	Whyte *et al*., 2009
New Zealand Bay of Islands, Waitangi Bridge	Mean = 0.07	*P. canaliculus*	Whyte *et al*., 2009
Canada Barkley Sounds	Mean = 0.68 Mean = 0.93 Mean = 0.24 Mean = 0.53	*Mytilus spp.*	Bendell, 2009
Italy Northern Adriatic Sea	Range=1.12-2.41 Mean =1.59	*Modiolus barbatus*	Storelli and Marcotrigiano, 2001
Italy Gulf of Gaeta	Range = 2.10-3.21	*Mytilus galloprovincialis*	Conti and Cecchetti, 2003
Italy Adriatic Sea	0.12-0.29	*Mytilus galloprovincialis*	Martincic *et al.*, 1987
UK	Mean = 0.154 Range = 0.047 – 0.449		Food Standards Agency, 2005

Figure 2.4 Bar graph depicting the ranges and means (vertical line) of the various cadmium concentrations in mussels (µg/g wet weight)

2.4.5 Cadmium Concentration in Kidney

There are few data available on cadmium concentrations in kidneys for the countries studied. Much of the data includes smokers, or occupationally exposed persons.

The Casey *et al* (1982) study shows that there is a significant difference in cadmium concentrations between the cortex and medulla (Table 2.5). Therefore when comparing concentration one must be careful to associate which part of the kidney is used.

The concentrations of cadmium found in New Zealand kidneys are significantly larger than the studies from Canada and the UK (Table 2.5). In the McKenzie (1974) study the only smokers

were male. From the concentration difference (men = 153.85, women = 85), without taking any other factors into account, the higher cadmium concentrations in men may be due to the increase intake of cadmium in cigarettes.

The samples from Canada show that concentrations increase with increasing age (Table 2.5). Increases between age groups decreases with increase age. Cadmium concentrations from the UK study seem to be in line with the data from the Canadian study.

Cadmium concentrations in Italian kidneys could not be found.

Table 2.5 Cadmium concentrations found kidneys from autopsy samples take in New Zealand, Canada, Italy and the UK

Country	Gender	Age	Cadmium Concentration µg/g wet weight	Source
New Zealand	Both	0-79	Cortex median=111.81[a] Medulla median=32.66[b]	Casey *et al*., 1982
New Zealand	Men	22-84	Mean = 212.94[c]	McKenzie, 1974 Whole kidney included male smokers
	Women		Mean =117.65[d]	
Canada	Both	10-19	Mean = 4.48	Benedetti *et al*., 1999 Whole kidney Included smokers
		20-29	Mean = 12.58	
		30-39	Mean = 21.68	
		40-49	Mean = 27.54	
United Kingdom	Both	All ages	Mean = 19.1 Median = 16 range = 0.1-163	Lyon *et al*., 1999 Renal cortex Included smokers 3 subjects know to be coppersmiths

[a] convert dry weight to wet weight. 0.77 provided from study
86.1/0.77=111.81

[b] convert dry weight to wet weight. 0.79 provided from study
25.8/0.79=32.66

[c] convert dry weight to wet weight. 0.85 provided from study
181/0.85=212.94

[d] convert dry weight to wet weight. 0.85 provided from study
100/0.85=117.65

2.4.6 Average Cadmium Intake

Table 2.6 shows the average cadmium intake levels for the four countries. The countries can be easily divided into two categories, those with comparatively lower cadmium intake and those with higher (Table 2.6 Figure 2.5). Italy and the UK fall into the lower category while New Zealand and Canada have the higher, with New Zealand having the highest average cadmium intake (Figure 2.5).

The Italian intake value is for Pavia in Northern Italy, not the whole country.

Table 2.6 Average daily cadmium intake (mg/day) in populations from New Zealand, Canada, Italy and the UK

Country	Year	Mean Cadmium Concnetration mg/day	Age/sex	Source
New Zealand	1997/1998	0.032[a]	19-24 yr Male	Vannoort and Thomson, 2005
Canada	1993-1997	0.028[b]	20-39 yr Male	Health Canada 2010
Italy Pavia	2004	0.014[c]		Turconi *et al.*, 2009
UK	1997	0.012		Food Standards Agency, 2009

[a] calculated from 2.8 µg/kg body weight/week
2.8/1000 x 80 / 7 = 0.032

[b] calculated from 0.394 µg/kg body weight/day. Body weight from personal communication (Dabeka B, 2010)
0.394/1000 x 71.3 = 0.028

[c] calculated from 13.6 µg/day
13.6/1000=0.012

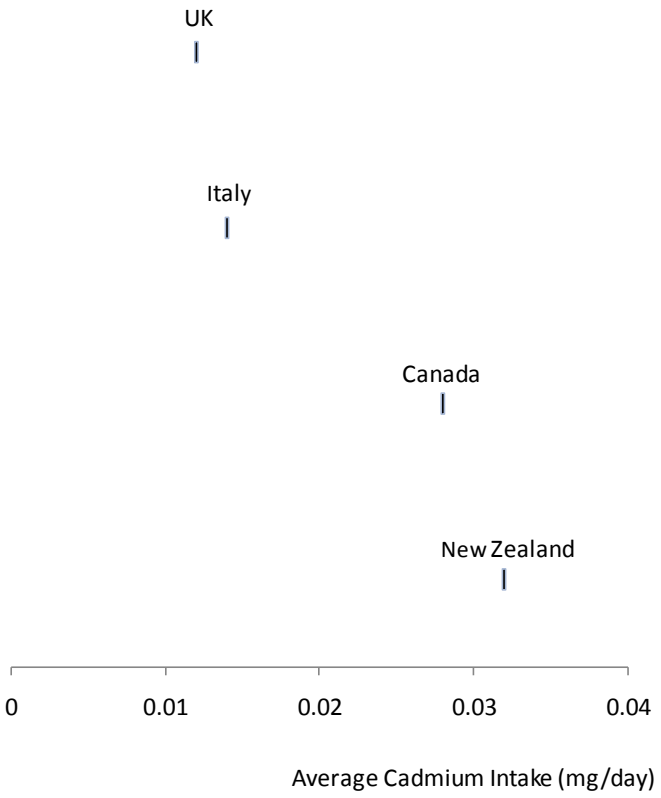

Figure 2.5 Average Cadmium intake levels for New Zealand, Canada, Italy and the UK

2.4.7 No Observable Effect Level

Experiments in mice showed that Cadmium was immunosuppressive at doses above 0.025 mg/day, based on $CdCl_2$ administered *ad lib* in water at 5 µg/mL (Blakley, 1985) and mouse daily water consumption of 4.9 ± 0.6 mL/day (Blakley, 1988).

Calculation:
5ppm = 5µg/ml

Convert µg/ml to mg/ml:
5/1000 = 0.005

0.005 x 4.9 = **0.025**

This calculated NOEL shows that both New Zealand and Canada currently have dietary intakes of cadmium that exceed the NOEL.

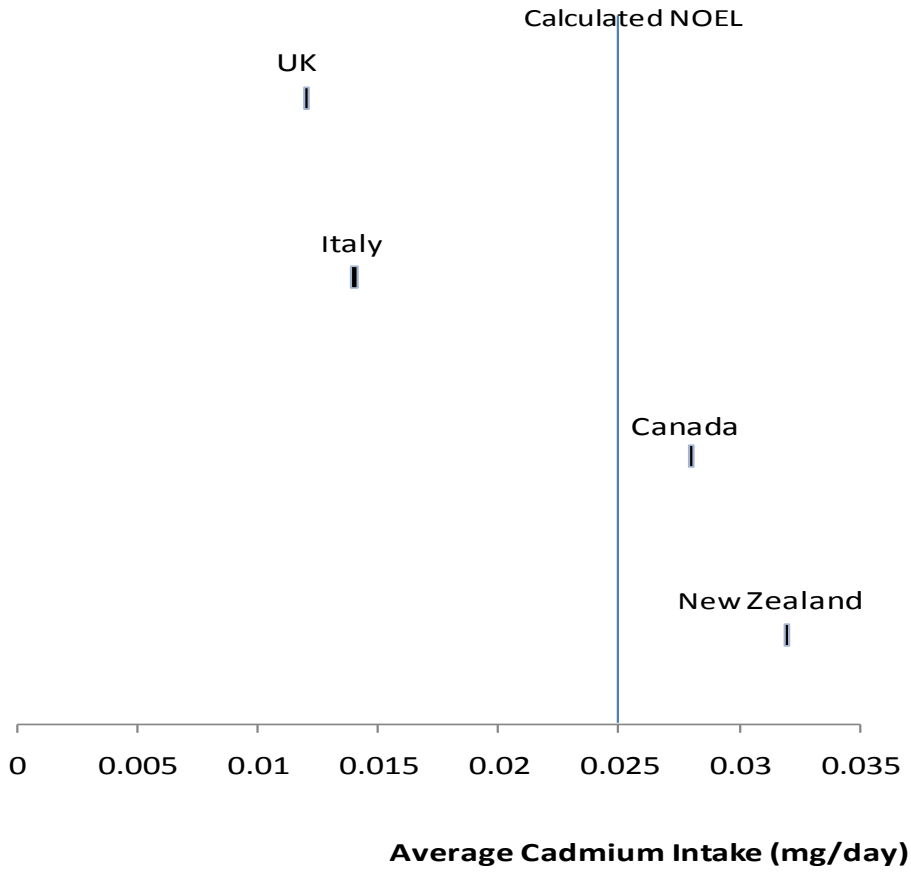

Figure 2.6 the placement of the No Observable Effect Level (NOEL) on relation to the average cadmium intake levels for New Zealand, Canada, Italy and the UK

2.5: Discussion

Interestingly New Zealand has some of the lower soil and oceanic cadmium concentrations investigated of the countries in this study. However, cadmium concentrations in found oysters, particularly *O. lutaria*, and levels observed in human kidney samples were relatively high. In addition, New Zealanders average daily intake of cadmium was the highest seen when comparing Canada, Italy and the UK.

2.5.1 Land and Aquatic Based Cadmium

As mentioned earlier various anthropogenic influences can increase cadmium levels in the environment, and these influences vary from country to country.

High cadmium concentration found in the soils for the Hamps and Manifold, in the UK can be explained by the historic mining in the area (Bradley and Cox, 1986). The Hamps and Manifold are rivers, soil samples were collected near active floodplains of both rivers. The catchments of these rivers were mined for copper, zinc and lead. Mining operations were in the catchment of the Manifold was significantly larger than that of the Hamps. Mining ceased in the Hamps in 1868, while mining continued in the Manifold until 1904. This difference in scale of mining activity can clearly be seen in the increased ranged and mean cadmium concentrations found in the Manifold (Figure 2.1). These results clearly demonstrate the impact that mining can have on cadmium soil concentrations of the surrounding environment.

Soils surrounding refineries, smelters and factories that process cadmium and cadmium related ores, such as zinc and copper also contain elevated concentrations of cadmium (Little and Martin, 1972). Both studies conducted in Canada looked at regions where mining and smelting facilities occurred (McIlveen, 2000, Jones, 2003). The cadmium concentrations in these soils were within that of New Zealand, Italy and the UK.

Combustion of fossil fuels can also increase cadmium levels in soils. This can be seen in the relatively high concentrations of cadmium found in soil samples surrounding a major highway in Yorkshire (Akbar *et al*., 2006). Generally the levels of contamination decrease with increasing distance from the source of contamination (Little and Martin, 1972, Muskett, 1979, Akbar *et al*., 2006). Airborne cadmium particles have been found to travel from western

European countries to the UK (Williams and Harrison, 1984). However, it is unlikely that any significant amount would accumulate in any one place by this method.

New Zealand has one of the lower mean soil cadmium concentrations (0.35mg/kg), however the range observed in New Zealand is relatively large, only the UK sites having larger ranges. While New Zealand is a volcanic country, the large range observed can principally be attributed to agricultural and horticultural practices. Mean soil cadmium concentration is approximately double the background levels (0.35mg/kg and 0.16 mg/kg, respectively) (Taylor, *et al*., 2007). Dairying soils contain the highest mean concentrations, 0.73 mg/kg of cadmium. The ever increasing dairying industry has the potential could increase this concentration and the national mean. Other soils which have higher than the average cadmium soil levels include kiwifruit (0.71 mg/kg), berries (0.68 mg/kg), orchards (0.66 mg/kg), market gardening (0.46 mg/kg) and dry stock pasture (0.40 mg/kg) (Taylor, *et al*., 2007).

In agricultural settings the spraying of sewage sludge from dairy sheds, the application of fertilisers and irrigation to increase pasture growth all impact cadmium levels in the soils.
It is well known that nitrates and phosphates from farmland can pollute rivers and oceans causing eutrophication (Cadmium Working Group, 2008). The Cadmium levels in oysters from Mahurangi catchment are believed to be influenced by the runoff and leaching of fertilisers from farmland up stream (Butler and Timperley, 1996). It is unlikely that the movement of cadmium from land to aquatic environments can ever be completely inhibited. However there are measures that can be taken to reduce this movement. The New Zealand fertiliser industry has implemented a volunteer maximum concentration of cadmium in phosphate fertiliser (Cadmium Working Group, 2008). This reduction on cadmium concentrations has been achieved by sources phosphate rocks which are low in cadmium. While this standard has only been applied in New Zealand, many other countries have switched to using low cadmium containing phosphate rocks for fertiliser production. Farming methods are constantly being revised and improved to help reduce the loss of nutrients and associated compounds (such as cadmium) from soils into surrounding environments.

It is difficult to directly compare cadmium soil concentrations between the different countries due to the differences in natural and anthropogenic sources of contamination. Italy has limited mining and processing of cadmium, and cadmium associated ores however it is a highly active

volcanic country. The lack of anthropogenic sources of cadmium pollution may be the reason for Italy's relatively low levels of cadmium in the soil. It is interesting to note that with the exception of the studies in the UK, cadmium concentrations in soils of Italy and Canada all overlap, and are within the range of New Zealand.

The overall concentration of cadmium in soils in significant, however more important is cadmium mobility. The two major factors influencing cadmium mobility in soils are acidity and organic matter content. (Olsson, *et al*.,2002). Mobile cadmium can be more readily absorbed by plants and lost to aquatic environments to be accumulated by animals, such as shellfish. Cadmium concentrations in the surface waters of the open ocean are relatively similar (Figure 2.2). However cadmium concentrations in coastal and estuarine waters can be heavily influenced by land based anthropogenic activities.

2.5.2 Cadmium in shellfish

Shellfish have the ability to accumulate and concentrate metals from the surrounding water, this is one of the reasons they have been used worldwide for many years to monitor water quality. Cadmium is one such metal that shellfish, particularly oysters and scallops concentrate. While cadmium does not physically appear to harm the shellfish, there has been some concern about dietary intake levels of cadmium for humans, especially of oysters and scallops.

Cadmium levels in shellfish can vary between and within species (Table 2.3 and 2.4). Consequently when discussing relative levels of cadmium (and any other metal) in different species of shellfish caution should be applied. Cadmium concentrations can also vary within different tissues of the animal and with varying life stages, as seen in *C. gigas* in Mahurangi catchment. Cadmium is usually accumulated in the viscera (guts) of shellfish and therefore removal of the guts would decrease cadmium intake from these animals. Within species cadmium levels can also vary with differing life stage, as shown by the difference in mean and range between pre and post spawning oysters in the UK (Food Standards Agency, 2005).

Not all cadmium enrichment in shellfish is caused by anthropogenic influences. In a study by Whyte *et al*. (2009) a hydrothermal vent was found to be the source of high levels of cadmium in greenshell mussels in Urapukapuka Island, this site was considered to be the control site. Italy is a volcanic country, indeed high cadmium concentrations in shellfish from Italy may be

the result of naturally high occur cadmium concentration in the environment, where anthropogenic sources cannot be established.

Environmental and physiological condition plays an important role in the uptake and accumulation of cadmium in shellfish. The length of time submerged and the presence of food influence the time shellfish spend filtering (Cheng and Gobas, 2007). In aquaculture animals which in the 'wild' may be exposed to tidal influences and therefore periods of time out of water, can be constantly submerged. Indeed as found by Cheng and Gobas (2007) this may increase the period of time spent feeding, increased cadmium intake, allowing for higher cadmium concentrations in aquaculture shellfish than they would in the wild.

The physiology of cadmium and of shellfish plays an important part in the uptake of cadmium by these shellfish. Cadmium bound to sediments is taken up and digested more readily than other forms of cadmium (Ray, 1984). Shellfish can also accumulate cadmium through the ingestion of phytoplankton (Lekhi *et al*., 2008). Once absorbed cadmium can be accumulated in tissue (mainly the viscera) and/or excreted as pseudofaeces. Metal-binding proteins can vary between species. In New Zealand the species *O. lutaria* contains a protein similar to metallothionein however the same protein was not detected in *C. glomerata (*Nordberg *et al*., 1986*).* Increased environmental cadmium concentrations for *O. lutaria* may mean it has developed metallothionein to detoxify cadmium. The form in which cadmium is dealt with in oysters may also explain why people that consume large quantities of high cadmium oysters do not necessarily have increased cadmium body burdens (McKenzie-Parnell *et al*., 1988).

2.4.3 Cadmium Intake and NOEL

When comparing cadmium concentrations in kidneys, it is important to compare the same part of the kidney. Cadmium concentrations in the kidney cortex are higher than the medulla (Casey, *et al*., 1982). Factors such as occupational exposure and smoking habits can significantly influence cadmium burden. The large range observed in the UK study can be explained by the inclusion of kidney samples from three coppersmiths.

Age is an important factor when considering the body burden of cadmium. As mentioned previously cadmium concentrations increase over a life time, starting a zero at infancy and rising until around the age of 60 when they can start to decrease (Lyon, *et al*., 1999). The decreases in cadmium after the age of 60 is thought to be due to a decrease in protein synthesis

of metallothionein related to age (Nordberg and Nordberg, 2009). It is important to take care when interpreting cadmium results in kidneys, as many factors can influence cadmium burden.

Cadmium levels observed whole kidneys in New Zealand (McKenzie, 1974) study far exceed the results shown for whole kidneys in Canada. A similar conclusion can be drawn from the renal cortex samples from Casey *et al* (1982) and Lyon *et al* (1999) where the median for New Zealand is at the upper range of the UK range which included coppersmiths. The large differences in cadmium contents are interesting. Both New Zealand studies are relatively old, however it is unlikely that cadmium concentrations in kidneys would decrease so significantly in 20-30 years to be in line with the levels observed in the UK and Canada studies.

Not surprisingly dietary cadmium intakes vary. Both UK and New Zealand dietary studies have shown decreases in daily intake levels in the last decade or so (Food Standards Agency, 2009; Vannoort and Thomsom, 2005). Reasons for these decreases may result from decreases in environmental levels due to a reduction in emissions from mining activities and improved processing techniques of the metal. Cadmium from the use of phosphate ferilisers has been decreased by sourcing low cadmium phosphate rocks and reduced application. A decrease in the consumption of cadmium rich foods, such as offal may go some way to explaining the decrease in daily intake.

Many factors influence cadmium absorption, as mentioned previously calcium and iron deficiencies can increase cadmium uptake. Generally women are associated with calcium and iron deficiencies. Indeed this has resulted in women generally having higher cadmium burdens than men (Benedetti *et al*., 1999). Some populations and ethnicities have diets which consume more cadmium rich food, such as rice, seafood, and animals that are slaughtered older such as horses, moose and caribou and mink. Canada had the second highest dietary intake of cadmium. Interestingly Canada is also one of the world's largest producers of cadmium. In addition, dietary customs of indigenous people such as of the First Nation's, Inuit and Metis (Gamberg *et al*., 2005) who regularly consume the offal of deer, moose, caribou and mink. All of which are known to be rich sources of cadmium (Gamberg *et al*., 2005, Arnold *et al*., 2006). Concerns have been raised about the dietary exposure to cadmium due to regular consumption of these animals offal. Cadmium burdens in these animals are relatively high due to the increase in age in which they are generally slaughtered, therefore consumption of the liver and kidney should be carefully monitored.

Maori, Pacific Islanders and Asians consume more seafood than other ethnic groups in New Zealand (Whyte *et al*., 2009). Although wild shellfish are grown throughout New Zealand, most commercially harvested shellfish are grown in a limited number of locations that considered 'clean' environments and are subject to environmental monitoring (Whyte *et al*., 2009) from anthropogenic sources. However even 'clean' locations can lead to naturally increased levels of cadmium levels in shellfish, as shown in *O. lutaria*. All these factors must be taken into account when comparing cadmium concentrations.

Of the studies investigated New Zealand had relatively low environmental cadmium concentrations in soil and oceanic waters, it is therefore interesting that New Zealand had the highest average daily intake of cadmium, this may indicate that what cadmium is in the environment in highly mobile and can easily be taken up in the food chain. The NOEL calculated using immunosuppressive level of $CdCl_2$ administered in the drinking water if mice. Assuming that the mouse immunosuppressive NOEL is of the same order as the human NOEL, Italy and the UK are below whereas Canada and New Zealand are above. these data while need to be interpreted with caution suggest that dietary exposure in Canada and more so in New Zealand might be immunosuppressive whereas exposure in the UK and Italy are less likely to result in immunosuppression.

Chapter 3: The Norovirus and Cadmium Conclusion

This study found no statistically significant relationship between Norovirus outbreaks and rainfall. This is interesting as numerous studies have found increased levels of Norovirus virions present in the aquatic environment after periods of heavy rainfall, and during these periods the presence of Norovirus has been confirmed in shellfish. Norovirus virirons can be found in shellfish all year round. This is shown in the tuatua and cockle data supplied by the Christchurch Regional Councils data on the presence of Norovirus virions in tuatua and cockles.

In addition to harbouring Norovirus virions shellfish also to concentrated relatively large levels of cadmium. New Zealand oysters especially, the bluff oyster *O. lutaria,* contain some of the highest cadmium concentrations observed in unpolluted waters in the world. Cadmium concentrations observed in shellfish can be influence by natural sources of cadmium (e.g. the hydrothermal sea vent in Urapukapuka, Bay of Islands New Zealand). In addition anthropogenic cadmium sources (e.g. mining, smelting, industrial processing of cadmium and the application of phosphate fertilisers and sewage sludge to land), can enter the aquatic environment to consequently be ingested by shellfish. The effects of increased cadmium exposure to humans are numerous however it is cadmiums immunosuppressive ability that this study focuses on.

The most interesting conclusion from this study is that when Norovirus outbreaks and cadmium intakes are both taken into consideration, shellfish are a common exposure mechanism for both. Increased cadmium intake and exposure to Norovirus may be enhanced in individuals who consume wild shellfish as opposed to supermarket purchased. Withstanding removing oysters from the diet completely the removal of the viscera and appropriate cooking techniques have the potential to decrease the risk of intake of high cadmium levels, and the ingestion of enteric viruses, such as Norovirus.

Individuals who consume large amounts of shellfish may have a larger risk of increased cadmium intake and exposure to infection from Norovirus. New Zealand has the highest rate of foodborne infections in the western world (Figure 1). Shellfish are an important customary food for Māori, and are more likely to be collected from the wild than purchased at a supermarket. It is unlikely that the dietary intake from oysters alone is enough to have

immunosuppressive effects, however when all dietary sources of cadmium taken into consideration, New Zealand has the highest average daily intake of cadmium out of the four countries investigate. In addition this intake level is also above the calculated NOEL. This raises an important question: is New Zealand high dietary cadmium intake suppressing our immune systems and allowing for a higher rate of foodborne illness to occur?

Future Research.

The issue of immunosuppression from increased cadmium intake needs to be investigated further. The potential implication of elevated dietary cadmium intakes contributing to immunosuppression is large and is an issue that has not been investigated at a large level. A more comprehensive study into the cadmium concentrations in kidneys of New Zealanders should be conducted, to further investigate the high cadmium concentrations found in New Zealanders kidneys. Much of the work done on increased cadmium intake has looked at the effect to the kidney and at what levels these happen. Little work has been done on the effects of dietary cadmium intake on the immune system in human.

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Appendix 1

Monthly raw data for reported outbreaks and rainfall for Auckland, Wellington, Christchurch, Dunedin and the Nation

Auckland Norovirus Outbreak Data

	2000	2005	2006	2007	2008	2009
Jan	8	6	0	9	2	2
Feb	1	4	2	3	1	2
Mar	2	4	9	5	4	1
Apr	0	1	5	3	10	4
May	1	2	2	5	6	3
Jun	0	0	2	6	8	11
Jul	2	1	5	10	10	9
Aug	2	1	9	7	4	8
Sep	0	1	3	7	1	10
Oct	2	4	5	10	5	9
Nov	3	3	1	7	5	17
Dec	4	6	2	7	3	4
TOTAL	26	33	45	79	59	80

Auckland Average Rainfall Data (mm)

	2005	2006	2007	2008	2009
Jan	21	77	75	19	38
Feb	57	7	6	70	132
Mar	33	52	164	34	40
Apr	30	183	66	146	56
May	125	183	23	88	162
Jun	116	112	109	173	141
Jul	138	77	281	258	134
Aug	42	114	125	188	88
Sep	74	56	90	51	113
Oct	177	180	122	98	119
Nov	45	89	64	54	35
Dec	78	50	83	105	123

Wellington Norovirus Outbreak Data

	2000	2005	2006	2007	2008	2009
Jan	1	1	3	3	1	1
Feb	1	3	0	1	0	0
Mar	1	0	2	0	1	2
Apr	0	0	3	2	0	2
May	0	0	2	2	3	6
Jun	0	2	1	0	1	8
Jul	0	0	1	0	1	4
Aug	1	0	0	1	2	4
Sep	0	1	0	3	2	3
Oct	0	1	0	3	1	1
Nov	0	0	1	3	4	0
Dec	2	1	3	2	1	1
TOTAL	6	9	16	20	17	32

Wellington Average Rainfall Data (mm)

	2005	2006	2007	2008	2009
Jan	106	55	88	43	29
Feb	34	82	15	48	153
Mar	121	88	42	117	34
Apr	43	93	61	163	97
May	158	106	54	52	157
Jun	52	129	85	132	66
Jul	115	232	138	252	74
Aug	30	188	74	178	140
Sep	45	40	52	102	74
Oct	83	174	168	96	177
Nov	26	185	41	64	78
Dec	73	77	74	116	66

Christchurch Norovirus Outbreak Data

	2000	2005	2006	2007	2008	2009
Jan	0	2	3	1	0	1
Feb	0	0	0	10	1	0
Mar	0	1	1	2	0	1
Apr	0	1	4	2	3	2
May	0	0	2	3	4	0
Jun	0	4	4	5	3	0
Jul	1	0	1	0	1	2
Aug	0	2	1	4	1	2
Sep	1	2	3	4	1	3
Oct	0	0	6	7	2	8
Nov	1	0	4	8	1	4
Dec	1	0	3	7	1	3
TOTAL	4	12	32	53	18	26

Christchurch Average Rainfall Data (mm)

	2005	2006	2007	2008	2009
Jan	25	32	29	12	27
Feb	21	52	30	120	67
Mar	39	53	34	20	24
Apr	51	65	62	32	52
May	77	107	16	60	151
Jun	17	96	64	86	22
Jul	29	51	73	164	38
Aug	13	103	37	96	48
Sep	33	6	30	37	28
Oct	48	86	74	27	83
Nov	35	68	30	8	19
Dec	40	117	54	56	38

Dunedin Norovirus Outbreak Data

	2000	2005	2006	2007	2008	2009
Jan	0	0	1	1	0	0
Feb	0	0	2	0	1	1
Mar	0	0	2	2	0	2
Apr	1	0	2	1	0	1
May	0	0	1	1	1	0
Jun	0	0	1	0	1	0
Jul	0	0	1	1	2	0
Aug	0	0	0	1	5	2
Sep	0	0	0	1	0	0
Oct	0	1	0	2	4	6
Nov	0	0	2	1	1	4
Dec	0	0	0	1	0	4
TOTAL	1	1	12	12	15	20

Dunedin Average Rainfall Data (mm)

	2005	2006	2007	2008	2009
Jan	51	61	28	36	38
Feb	74	36	18	40	118
Mar	60	30	32	50	34
Apr	29	108	32	43	27
May	39	39	13	52	101
Jun	27	42	58	29	13
Jul	19	20	92	93	35
Aug	11	29	19	37	16
Sep	33	12	34	48	37
Oct	37	39	5g7	24	38
Nov	36	75	25	26	13
Dec	92	99	70	72	40

National Norovirus Outbreak Data

	2000	2005	2006	2007	2008	2009
Jan	9	12	12	18	4	7
Feb	2	7	9	18	5	3
Mar	4	6	23	16	5	14
Apr	1	3	16	9	15	21
May	1	3	12	12	18	19
Jun	0	7	10	13	15	31
Jul	4	1	10	12	18	32
Aug	4	3	12	13	15	24
Sep	1	4	12	21	9	31
Oct	2	6	16	29	22	36
Nov	5	3	11	23	18	34
Dec	7	7	15	23	10	19
Total	40	62	158	207	154	271

National Average Rainfall Data

	2005	2006	2007	2008	2009
Jan	65	105	80	52	52
Feb	74	67	35	78	146
Mar	118	87	96	78	53
Apr	46	166	66	128	92
May	141	117	62	78	145
Jun	85	124	127	126	104
Jul	122	123	143	207	117
Aug	59	113	97	151	124
Sep	84	58	82	98	94
Oct	125	127	144	107	135
Nov	62	150	45	84	63
Dec	115	99	104	117	98

Appendix 2

Seasonal raw data for reported outbreaks for Auckland, Wellington, Christchurch, Dunedin and the Nation

Auckland Norovirus Outbreaks Data

	2005	2006	2007	2008	2009
Autumn	63	139	84	89	86
Winter	99	101	138	207	121
Spring	99	108	92	68	89
Summer	39	54	44	57	92

Wellington Seasonal Norovirus Outbreaks

	2005	2006	2007	2008	2009
Autumn	107	96	52	110	96
Winter	66	183	99	187	93
Spring	51	133	87	87	109
Summer	70	70	60	55	99

Dunedin Seasonal Norovirus Outbreaks

	2005	2006	2007	2008	2009
Autumn	40	59	26	48	54
Winter	19	30	56	53	21
Spring	35	42	39	33	30
Summer	63	63	48	49	76

National Seasonal Norovirus Outbreaks

	2005	2006	2007	2008	2009
Autumn	12	51	37	38	54
Winter	11	32	38	48	87
Spring	13	39	73	49	101
Summer	19	28	51	32	20

Appendix 3

Raw data supplied by Christchurch City Council for norovirus contamination in cockles

Month Received	Site	NoV GI (log RTPCRU /gram shellfish guts)	Relative Level of GI NoV	NoV GII (log RTPCRU /gram shellfish guts)	Relative Level of GII NoV
Apr-08	Pleasant Pt Jetty	1.8	Low	1.8	Low
Apr-08	Plover Street	1.8	Low	1.8	Low
Apr-08	Causeway	1.6	Low	2	Moderate
Apr-08	Heathcote Mouth	1.8	Low	2.1	Moderate
Apr-08	Discharge	1.8	Low	1.8	Low
Apr-08	SCE Site 6	N/D	N/D	N/D	N/D
Apr-08	SCE Site 7	N/D	N/D	N/D	N/D
Apr-08	SCE Site 8	N/D	N/D	N/D	N/D
Jul-08	Pleasant Pt Jetty	4.3	Ex. high	4	Ex. high
Jul-08	Plover Street	3.7	Very high	3.5	Very high
Jul-08	Causeway	4.2	Ex. high	3.7	Very high
Jul-08	Heathcote Mouth	4.3	Ex. high	3.9	Very high
Jul-08	Discharge	4	Ex. high	3.6	Very high
Jul-08	SCE Site 6	N/D	N/D	N/D	N/D
Sep-08	Pleasant Pt Jetty	3	High	3.5	Very high
Sep-08	Plover Street	2.5	High	3.5	Very high
Sep-08	Causeway	2.8	High	3.6	Very high
Sep-08	Heathcote Mouth	3	High	3.5	Very high
Sep-08	Discharge	3.1	Very high	3.3	Very high
Sep-08	SCE Site 6	1.6	Low	1.9	Low
Dec-08	Pleasant Pt Jetty	1.3	Low	3.6	Very high
Dec-08	Plover Street	1.6	Low	3.4	Very high
Dec-08	Causeway	N/D	N/D	3	Very high
Dec-08	Heathcote Mouth	1.3	Low	3.6	Very high
Dec-08	Discharge	1.6	Low	3.2	Very high
Dec-08	SCE Site 6	N/D	N/D	N/D	N/D
Mar-09	Pleasant Pt Jetty	N/D	N/D	1.9	Low
Mar-09	Plover Street	N/D	N/D	1.6	Low
Mar-09	Causeway	N/D	N/D	1.9	Low
Mar-09	Heathcote Mouth	N/D	N/D	2.2	Moderate
Mar-09	Discharge	N/D	N/D	2.3	High
Mar-09	SCE Site 6	N/D	N/D	N/D	N/D
Jun-09	Pleasant Pt Jetty	3.0	Very High	3.4	Very High
Jun-09	Plover Street	2.3	High	2.7	High

Table continued...

Jun-09	Causeway	2.3	High	2.8	High
Jun-09	Heathcote Mouth	2.8	High	3	Very High
Jun-09	Discharge	2.8	High	3.1	Very High
Jun-09	SCE Site 6	N/D	N/D	1.6	Low
Sep-09	Pleasant Pt Jetty	2.6	High	3.1	Very High
Sep-09	Plover Street	2.0	Moderate	2.9	High
Sep-09	Causeway	2.4	High	3	High
Sep-09	Heathcote Mouth	2.3	High	3.3	Very High
Sep-09	Discharge	2.5	High	3.1	Very High
Sep-09	SCE Site 6	N/D	N/D	N/D	N/D

N/D-not detected, Ex. High – extremely high

Saltwater Creek Estuary data not used in results due to limited detection

Appendix 4

Raw data supplied by Christchurch City Council for norovirus contamination in tuatua

		Norovirus GI		**Norovirus GII**	
Rockinghorse Road		RT-PCR	#/gm	RT-PCR	#/gm
	May-07	-ve		**+ve**	
	Jun-07	-ve		**+ve**	
	Aug-07	**+ve**	B/Q	**+ve**	**400**
	Sep-07	**+ve**	B/Q	**+ve**	B/Q
	Oct-07	-ve		**+ve**	B/Q
	Nov-07	-ve		**+ve**	B/Q
	Dec-07	-ve		**+ve**	B/Q
	Jan-08	-ve		-ve	
	Feb-08	**+ve**	B/Q	**+ve**	B/Q
	Mar-08	**+ve**	B/Q	**+ve**	B/Q
	Apr-08	**+ve**	B/Q	**+ve**	B/Q
	May-08	**+ve**	3540	**+ve**	1680
	Jun-08	**+ve**	1170	**+ve**	600
	Jul-08	**+ve**	760	**+ve**	1160
	Aug-08	**+ve**	830	**+ve**	700
	Sep-08	**+ve**	N/D	**+ve**	300
	Oct-08	-ve	N/A	**+ve**	160
	Nov-08	-ve	N/A	**+ve**	260
	Dec-08	-ve	N/A	**+ve**	1350
Sumner Surf Club	**May-07**	-ve		**+ve**	
	Jun-07	-ve		**+ve**	
	Aug-07	**+ve**	B/Q	**+ve**	**242**
	Sep-07	**+ve**	B/Q	**+ve**	B/Q
	Oct-07	-ve		**+ve**	B/Q
	Nov-07	-ve		**+ve**	B/Q
	Dec-07	**+ve**	B/Q	**+ve**	B/Q
	Jan-08	-ve		-ve	
	Feb-08	**+ve**	B/Q	**+ve**	B/Q
	Mar-08	-ve		**+ve**	B/Q
	Apr-08	**+ve**	B/Q	**+ve**	B/Q
	May-08	**+ve**	1660	**+ve**	580
	Jun-08	**+ve**	2170	**+ve**	430
	Jul-08	**+ve**	730	**+ve**	270
	Aug-08	**+ve**	200	**+ve**	500
	Sep-08	**+ve**	140	**+ve**	700
	Oct-08	**+ve**	N/D	**+ve**	670
	Nov-08	**+ve**	N/D	**+ve**	300
	Dec-08	**+ve**	N/D	**+ve**	1150

Table continued...

		Norovirus GI		**Norovirus GII**	
		RT-PCR	#/gm	RT-PCR	#/gm
Jellicoe Street	**May-07**	**+ve**		**+ve**	
	Jun-07	**+ve**		**+ve**	
	Aug-07	-ve	B/Q	**+ve**	**100**
	Sep-07	**+ve**	B/Q	-ve	
	Oct-07	-ve		**+ve**	B/Q
	Nov-07	-ve		**+ve**	B/Q
	Dec-07	-ve		-ve	
	Jan-08	-ve		-ve	
	Feb-08	**+ve**	B/Q	**+ve**	B/Q
	Mar-08	**+ve**	B/Q	**+ve**	B/Q
	Apr-08	**+ve**	B/Q	**+ve**	B/Q
	May-08	**+ve**	2220	**+ve**	1430
	Jun-08	**+ve**	990	**+ve**	770
	Jul-08	**+ve**	B/Q	**+ve**	175
	Aug-08	**+ve**	230	**+ve**	700
	Sep-08	**+ve**	500	**+ve**	3700
	Oct-08	-ve	N/A	**+ve**	360
	Nov-08	**+ve**	N/D	**+ve**	N/D
	Dec-08	-ve	N/A	**+ve**	150

Publications

In press

Conn, A and Shaw, I. Could Cadmium-induced immunosuppression explain the high incidence of food borne illness in NZ? *New Zealand Journal of Environmental Health*

Could Cadmium-induced immunosuppression explain the high incidence of food borne illness in NZ?

Ailsa Conn & Ian Shaw*

Abstract

It is difficult to understand why New Zealand has one of the highest incidences of food borne illness (e.g. campylobacteriosis) in the developed world. New Zealand also has high environmental cadmium levels due to its geological origins with concomitantly high dietary cadmium intakes particularly from filter feeding shellfish. Meta-analysis of published cadmium exposure data from the UK, Canada and Italy corroborate the geological origins theory for dietary cadmium intake. Cadmium is immunosuppressive and it is postulated that constant exposure to cadmium in the diet of New Zealanders results in low grade immunosuppression which in turn leads to a decreased immune response to dietary pathogens with a concomitant increase in the incidence of food born illness.

Introduction

The immune status of a population is a key determinant in its susceptibility to bacterial and viral diseases. This is often considered in the context of cohort populations and their resistance to particular diseases due to their vaccination status or, perhaps, to larger populations exposed to a particular infection which might lead to post exposure immunity.

Dietary exposure to immunosuppressants is likely to have an impact on the response of a population to pathogens. Cadmium (Cd) is a well known immunosuppressant and is known to increase susceptibility to both bacterial and viral pathogens[1]. Cd exposure from country to country differs according to the particular country's geological origins. In this paper we explore published dietary and tissue levels of Cd in the populations of three countries (New Zealand, Italy and Canada) that have high environmental levels of Cd and compare them with the UK which has lower environmental Cd levels.

New Zealand and Italy are volcanic countries both having active volcanoes. Cd is released into the environment by volcanic eruptions and the weathering of volcanic rocks. Canada has volcanic origins, but no longer has an active volcano; therefore Cd leaches from rocks into the environment. In addition Canada has a significant metal mining industry and Cd salts are co-located with several commercially important metal ores, e.g. zinc, and so are released from sequestration in the earth's crust in the form of mine tailings. These tailings are eroded by rain to release Cd salts into the environment. The net effect of the geological origins and weathering processes in New Zealand, Italy and Canada is to release Cd salts in a bioavailable form into the environs of the contaminated rocks, soils and silts. The Cd salts can be bioaccummulated by food animals and represent a source of dietary Cd. In New Zealand mussels and oysters cultured or growing naturally adjacent to river estuaries are exposed to river silts derived from rocks and soils containing Cd. The fine silt particles are filtered out by the bivalves and accumulated in their tissues. This presents a significant dietary source of Cd to consumers of NZ mussels and oysters.

Other animals grazing on plants growing on soils derived from Cd-containing rocks are likely to bioaccumulate Cd, particularly in their livers and kidneys. However offals are not popular in New Zealand and therefore this is not likely to be a significant dietary source of Cd at a population level. On the other hand offals are popular in Italy, and moreover horse flesh (including offals) is consumed. Horses are often slaughtered for human consumption at a greater age than cattle, sheep and pigs and therefore since Cd is bioaccumulated tend to have higher tissue levels of Cd. This combined with shellfish consumption in Italy is likely to lead to a significant dietary intake of Cd.

Canada presents a quite different situation. Cd contamination from Zn mine tailings leaches into rivers and flows into the sea resulting in locally high Cd concentrations in aquatic environments adjacent to river estuaries. Oysters are farmed in these areas in Canada and therefore Cd is likely to enter the food chain via this route.

The UK's geological origins mean that Cd levels are low in rocks and minerals, in addition there is little or no metal mining and therefore mine tailings do not represent a potential source of Cd. For this reason the UK is used in this study as a low Cd environment.

In this paper we report results of meta-analysis of published data and explore whether human dietary Cd intake in New Zealand, Canada and Italy might be high enough to result in immunosuppression which, in turn, might make the population more susceptible to food borne pathogens.

Methods

The published literature was searched using GoogleScholar®, Web of Science® and Science Direct® for Cd concentrations in soil, sea water and mussel and/or oyster in samples from the UK, New Zealand, Italy and Canada. The data found were used to determine ranges and/or means for each sample class in each country. All data found were used in the meta-analysis.

An estimate of the immunosuppressive No Observable Effect Level (NOEL) of Cd was calculated from published studies in mice[2,3] in which $CdCl_2$ was administered *ad libitum* in drinking water. The approximate Cd dose received by the mice in the above studies was calculated using a published value for daily water consumption in mice[4].

Results

Environmental Cd levels in New Zealand, Italy, Canada and the UK

Cadmium levels in soils from New Zealand, Italy, Canada and the UK derived from published studies are shown in Table 1.

Table 1 here

It is clear from the data in Table 1 that levels of Cd in environmental samples are very variable. However the four countries have quite different, albeit overlapping, ranges which reflect the geological origins of the country and determine consumer exposure via food grown in that country or its marine environment.

Dietary exposure to Cd in New Zealand, Italy, Canada and the UK

The dietary intake of Cd in the UK was 0.012-0.018 mg/day (95^{th} percentile upper and lower bands)[18], in New Zealand the mean intake was 0.02 mg/day (calculated from NZ Total Diet Survey 2003/4[19]), in Pavia, northern Italy the intake range was 0.0014 – 0.033 mg/day (calculated from the Italian Total Diet Survey 1994/6[20]) and in Canada the intake range was 0.007 – 0.034 mg/day[21] . These data show overlapping ranges with the Cd intake range in Italy rising to a significantly higher value than in the UK, the NZ intake mean value is marginally greater than the upper UK band of the 95^{th} percentile and the Canadian intake range extends above the top of the UK 95^{th} percentile band, but at the lower end of the range it is only marginally above the UK lower 95^{th} percentile band (Fig. 1) ; this reflects the fact that Canada is not a volcanic country, but mines metals that release Cd into the environment and therefore presumably this leads to local contamination which might find its way into the human food chain.

Figure 1 near here

Experiments in mice showed that Cd was immunosuppressive at doses above 0.017 mg/day, i.e. the approximate No Observable Effect level (NOEL), based on $CdCl_2$ administered *ad lib* in water at 5 μg/mL[2] and and mouse daily water consumption of between 3.3 ± 0.2 mL/day[4] and 4.9 ± 0.6 mL/day[3] . There are no published data for immunosuppressive doses of Cd in humans, however occupationally exposed workers in Turkey showed no reduction in serum immunoglobulin levels[22]; this might be explained because they received a high doses over a short time and that chronic doses are necessary to result in immunosuppression. Assuming that the mouse immunosuppressive NOEL is of the same order as the human NOEL, the human Cd intake levels in the UK are just at or below the NOEL whereas the Italian exposure range extends far beyond the NOEL and the New Zealand intake value is just above the NOEL. These data suggest that dietary intake of Cd in New Zealand and Italy might be immunosuppressive whereas exposure in the UK is less likely to result in immunosuppression.

Discussion

Countries that are volcanic in nature (e.g. Italy and New Zealand) or that mine metals associated with Cd and so release the element from its sequestered state in the earth's crust (e.g. Canada) have greater consumer exposure to Cd than countries that do not have significant geothermal activity or Cd-associated metal mining (see Fig 1).

There are many reasons for varying incidences of food borne illness. Clearly exposure to pathogens in food is the most important, but the receptiveness of the consumer of the infected food to the particular pathogen is of significant importance. In this context the consumer's immunological status is a key factor that must contribute to the risk of infection. New Zealand's incidence of campylobacteriosis is declining, but remains one of the highest in the world[23, 24]. It is interesting that New Zealand's high campylobacteriosis incidence coincides with high Cd exposure. A cause and effect relationship is impossible to prove, but it is possible that Cd-induced immunosuppression is a risk factor for susceptibility to food borne

pathogens and that this, at least in part, accounts for New Zealand's high incidence of food borne illness.

Interestingly New Zealand mussels are a source of both Cd, norovirus and other faeces-borne pathogens because they are filter feeders and thus filter out Cd-containing silts from their aquatic environments and are also exposed to pathogens including norovirus and perhaps *Campylobacter* sp. in sewage from moored boats and sewage outfalls near to both wild mussel and aquaculture sites. While it is not suggested that the Cd contained in a meal of mussels would reduce the consumer's immunity against Norovirus and/or *Campylobacter* sp in the same meal, it is interesting to note that there is a risk that the immunosuppressant and pathogen might be co-ingested.

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Country	**[Cd] in sea water (0-5m) (ng/L)**	**[Cd] in soil (mg/kg)**	**[Cd] in oysters (µg/g tissue)**
New Zealand	[a]10.3 - 25.1 [b]2.3 - 6.4	[f]0.2 - 2.6	[o]0.12 - 5.9 [p]1.1 - 7.9 [q]0.12 - 5.0
Canada	[c] 51.7 - 93.0 [d]25.3 - 52.8	[g] 0 - 0.8 [h] 54 - 66	[r]1.17 - 3.57 [s]1.40 – 2.47
UK	[w]4 - 51 [x] 11 - 22 [y]13 - 81	[i]0.4 (mean) [j]2.14 (mean), range 0.39 - 6.57 [k]2.47 (mean), range 21.9 - 0.25	[t] 0.21 (mean) [u] 0.46 (mean)
Italy	[e]112 - 50,400 [v]115 ± 6 - 142 ± 4	[l] 39 (median), 12 – 100 (range) [m]0.22 (mean), range 0.08 - 0.60 [n] 0.50 (mean), range =0.10 - 1.20	No data found

Table 1
Published levels of Cd in soil, sea and oysters from New Zealand, Italy, Canada and the UK

[a] Off Otago Peninsula, South Island, calculated from Croot & Hunter (1998)[5]
[b]Foveaux Straight, South Island, calculated from Frew & Hunter (1995)[6]
[c] Deep Bay, Canada, calculated from Lekhi *et al* (2008)[7]
[d] lemmens, Canada, calculated from Lekhi *et al* (2008)[7]
[e] Naples Harbour, calculated from Rumolo *et al* (2009)[8]
[f] Samples from multiple sites, North and South Islands[9]
[g]Port Colborne Area[10]
[h] Smelter at Rouyn-Noranda Quebec[11]
[i]Calculated background level[12]
[j]Samples from mine contaminated area, The Hamps, Staffordshire[12]
[k] Samples from mine contaminated area, The Manifold, Staffordshire[12]
[l] Palermo, Italy[13]
[m]Rural area in Southern Italy[14]
[n] Industrial area in Southern Italy[14]

[o]*Ostrea lutaria* Wellington, Marlborough Sounds, Tasman Bay and Golden Bay[15]
[p] *Ostrea lutaria* Foveaux Strait and Stewart Island[15]
[q] *Crassostrea glomerata*, Upper North Island[15]
[r]Deep Bay, British Colombia[7]
[s] Lemmens inlet, British Colombia[7]
[t] *Crassostrea gigas*[16]
[u]*Ostrea edulis*[16]
[v] Tyrrhenian Sea, Italy[8]
[w] North Sea[17]
[x] English Channel[17]
[y] Irish Sea[17]

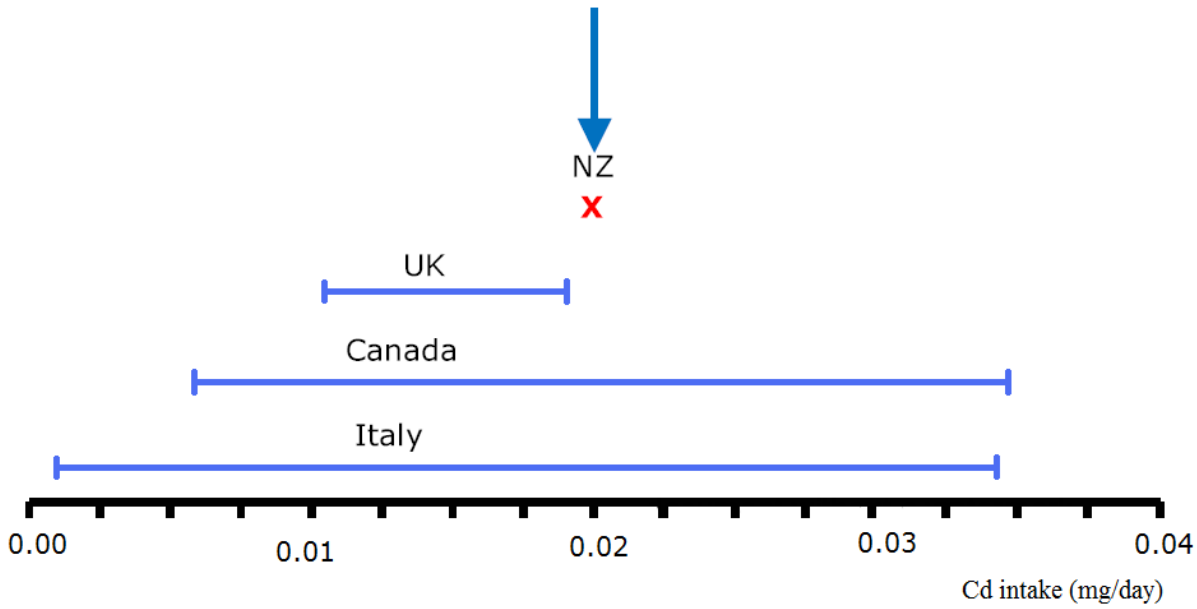

Figure 1

Intakes of Cd (mg/day) in New Zealand (mean value only), the UK, Canada and Italy. The bars show the ranges, except for the UK which is shown as upper and lower bands of the 95th percentile. Higher dietary intakes are associated with higher environmental levels of Cd